INFANT FORMULA WITH DOCOSAHEXAENOIC ACID, MATERNAL SMOKING, AND BODY MASS INDEX OF CHILDREN TO SIX YEARS OF AGE

BY

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Submitted to the graduate degree program in Dietetics and Nutrition and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master's of Science.

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ABSTRACT

Research findings have suggested no differences in growth among term infants fed DHA supplemented formulas. Studies have found maternal smoking decreases length and increases weight of children. No studies have analyzed maternal smoking and DHA supplementation on growth of term infants to age six.

The study aim was to determine if DHA supplementation in formula consumed from birth to one year and maternal smoking affects growth of children through six years. Anthropometric measures and maternal characteristics were collected at 16 study visits from birth to six years.

DHA supplementation increased weight-for-age and height-for-age but not BMI-for-age percentiles from two to six years. Maternal smoking during pregnancy increased weight-for-age, height-for-age, and BMI-for-age percentiles. Weight-for-age was not affected by age, however, BMI increased by 6.5% per year. Energy intake was not related to DHA or maternal smoking during pregnancy, suggesting effects on body size and fatness were unrelated to energy intake differences.

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Chapter 1

Introduction

Docosahexaenoic acid (DHA) is a long chain omega-3 polyunsaturated fatty acid essential for visual, cognitive, and central nervous system development in infants (1, 2). DHA was added to infant formulas in the United States in 2002 (3). A review of 13 randomized studies in preterm infants and 19 randomized studies in term infants determined omega-3 fatty acids may decrease growth of preterm and term infants, dependent on the experimental conditions, but the effects on growth appeared minimal and the clinical relevance was questioned (4). Three indicate DHA-supplemented formula fed to preterm infants improves weight gain (2, 5, 6). Six studies report the effects of DHA-supplemented formula fed to term infants and find no differences in weight or length (7-12).

We took advantage of a cohort of children who were evaluated every six months between birth and six years of age and who were provided one of four levels of DHA in formula to evaluate infant and child growth and BMI. We collected detailed records of dietary intake, formula tolerance, and anthropometrics and maternal characteristics including weight and smoking status. An earlier preliminary analysis of growth during the first four years of life in this cohort indicates that maternal smoking status does not affect weight-for-age percentiles but significantly affects length- and height-for-age percentiles in children through four years of age ($p=0.043$). Infants and children born to women who smoked were shorter and had higher BMI-for-age percentiles compared to those born to non-smoking women. No significant effects were found between infants

fed various percentages of DHA-supplemented formula and weight-for-length and BMI-for-age percentiles through age four (13).

Justification for Further Investigation

The study conducted at the University of Kansas Medical Center (13) is the only study that has tracked the longitudinal growth of infants fed DHA-supplemented formula and the effects of maternal smoking status through four years of age. Growth data are now available for children through six years of age, and we have the data to extend the evaluation to school age. Six studies have measured weight and length in term infants (7-12). This is the first dose-response study of DHA to determine the longitudinal growth of term infants. Because a high proportion of women smoked prior to or during pregnancy, the cohort offers the possibility to evaluate maternal smoking status and growth.

Statement of Purpose

The study has two primary objectives. The first objective is to determine if the concentration of DHA in a formula consumed from birth to 12 months of age affects growth from birth to six years of age, particularly in weight-for-length (for children younger than two years of age) and BMI-for-age (for children between two and six years of age). A second objective is to determine if maternal smoking during pregnancy affects BMI-for-age percentiles.

Primary Research Questions

1. Is DHA intake in infant formula consumed from birth to one year of age related to body mass index (BMI) percentiles (two to six years)?
2. Does maternal smoking during pregnancy affect BMI of children two to six years of age?

Secondary Research Question

Is energy intake related to BMI?

Chapter 2

Review of Literature

Infant and Child Growth Patterns

Infants and children grow at various rates from birth through adolescence. Immediately after an infant is born, he or she loses five to ten percent of his or her body weight (14). After the weight loss period, an infant's length and weight increase rapidly. It is normal growth for weight to double by four to six months of age. Typically, an infant's BMI increases and then decreases until four to five years of age (15, 16). By age four, a child's height is typically twice the birth length, and weight increases by six grams per day. At age five, a child should gain four to five pounds and grow two to three inches per year. School-aged children, six to twelve years old, will display differences in weight, height, and body composition. Variation in growth at all ages reflects a combination of genetics, nutrition, and exercise (14).

Stable growth continues from two to ten years of age, but the rate of growth and maternal weight may predict children becoming overweight or obese in the future. Children who have mothers who are overweight are at risk of becoming overweight, and low maternal weight is a predictor of underweight in children (17). A physiological adiposity rebound occurs when infant's and children's BMI rises for a long period of time. When BMI rises before the age of four or five years, early adiposity rebound occurs and results in increased risks for obesity as the child ages (15, 16).

Brune et al. (17) suggests BMI development occurs in phases. During an infant's first month of life, BMI sharply increases. There is another increase in BMI between eighteen months and five years of age (16). At this time, BMI increases in overweight

children and children experience another rapid weight gain, whereas children who are underweight remain at low weights and BMI declines. After age six, children with a high or low BMI typically remain stable (17).

Growth Charts for Infants and Children

Normal growth is exponential (14). The Centers for Disease Control and Prevention (CDC) created growth charts. The updated 2000 CDC growth charts are used by pediatricians, nurses, dietitians, and parents to assess and illustrate infant and child growth (18, 19). The charts are based on 82 million birth weights, 445,000 birth length measurements, and data from the National Health and Nutrition Examination Survey (NHANES), a cross-sectional survey of health and nutrition among people in the United States. Survey results from NHANES I (1971-1974), NHANES II (1976-1980), NHANES III (1988-1994), and the CDC Pediatric Nutrition Surveillance System (PedNSS) from 1975 to 1995 assisted in creating the growth curves (20).

The 2000 CDC growth charts include normal values for length or stature, weight, and BMI from birth through 20 years of age expressed as percentiles for each gender to account for differences in growth between males and females. The CDC charts measure head circumference-for-age and weight-for-length for males and females younger than two years of age (20). The charts also include measurements of weight-for-age and stature-for-age for boys and girls two years of age and older. BMI-for-age charts were added to the 2000 CDC growth charts for children and are used between ages two and 20 to assess weight-for-stature and to screen children at risk for low growth or excess body fat (21). BMI is calculated as body weight in kilograms (kg) divided by height in meters squared $(m)^2$ and is used to determine if children and adults are underweight, overweight,

or obese but can only be used starting at age two when height is more accurately measured (21).

To assist health professionals in determining growth risks, percentile ranges have been developed for weight-for-length and BMI-for-age. A weight-for-length or BMI-for-age percentile below the fifth percentile is considered underweight. Percentiles between the 5th and 85th are considered healthy. A percentile between the 85th and 95th percentile is considered overweight, and a percentile above the 95th percentile is classified as obese (21). The CDC growth charts are used for clinical assessments of children but are not used as the only diagnostic tool (18).

Diet and nutrition of the mother, infant, and child are determinants of growth and the likelihood for children to become overweight or obese in the future (22, 23). One nutrient that influences growth of infants and children is docosahexaenoic acid (DHA) (2, 5-12).

Docosahexaenoic Acid (DHA)

Definition and Mechanisms

Docosahexaenoic acid (DHA; 22:6n-3), an omega-3 long chain polyunsaturated fatty acid, is important for visual, cognitive, and central nervous system development in infants (1, 2). The omega-3 fatty acid alpha-linolenic acid (ALA; 18:3n-3) forms eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA) through desaturation by a microsomal enzyme system, and the omega-6 fatty acid linoleic acid (LA; 18:2n-6) forms arachidonic acid (AA or ARA; 20:4n-6) by desaturation (24). DHA affects cell membranes and signals between cells and genes that play a role in the growth of cells (2). DHA accumulates in the brain mainly during the last trimester of gestation

and in the infant's first two years of life, but this is influenced by the mother's DHA status and the DHA consumed postnatally (24, 25).

Dietary DHA Recommendations

The Institute of Medicine (IOM) set an Adequate Intake (AI) for ALA as 0.6% of the energy based on the amounts needed to prevent deficiency and promote growth and development (26). The IOM recommendations for the AI of ALA are 0.5 g/d for infants and 1.1 g/d for women (27). No requirement has been determined for DHA and EPA; however, the National Academies indicates 10% of the Acceptable Macronutrient Distribution Range (AMDR) for ALA can be consumed as DHA and EPA (26). Other experts recommend that pregnant and lactating women consume at least 200 to 300 mg of DHA per day (26). The same experts recommend that infant formula contain between 0.2 and 0.5% weight of total fatty acids with DHA higher than the level of ARA (26).

DHA in Infant Formula

In the past, infants have had to synthesize long chain polyunsaturated fatty acids (LC-PUFA) from fatty acids in infant formula. The European Society for Paediatric Gastroenterology and Nutrition suggested low birth weight infants be fed formula supplemented with alpha-linolenic and linoleic acids, similar to levels in breast milk. Conversion of ALA to DHA is limited during infancy; thus, alpha-linolenic acid in formula may not be needed for DHA (24, 28). DHA and ARA were added to infant formulas in the United States in 2002 (3). Because DHA and ARA levels are similar in breast milk and formula, infants who are formula fed should receive enough of these fatty acids for brain growth and development (6).

Effects of DHA on Growth

Research on formula supplemented with DHA and the effects on preterm infant growth is inconclusive. A review of 13 randomized studies in preterm infants and 19 randomized studies in term infants determined omega-3 fatty acids may decrease growth of preterm and term infants, dependent on the experimental conditions, but the effects on growth appeared minimal and the clinical relevance was questioned (4). O'Connor et al. (5), found that preterm infants fed formula supplemented with DHA and ARA until at least six months corrected age (CA) had higher growth and visual acuity development. Similarly, Innis et al. (6) found that preterm infants fed DHA and ARA gained more weight from 40 to 57 weeks post menstrual age compared to infants fed formula without DHA and ARA (34.7 grams per day (g/d) vs. 30.7 g/d, respectively; $p=0.004$); however, rate of weight gain of preterm infants fed formula with DHA (33.2 g/d) was not significantly different among infants fed formula with DHA and ARA or formula without DHA or ARA (6). From birth to four months of age, the weight-to-length ratio was higher in infants who consumed formula with DHA and ARA compared to formula with only DHA. However, at two months of age, infants who received DHA and ARA-supplemented formula had significantly higher body weight, length, and weight-to-length ratios compared to infants fed formula without DHA and ARA (6).

Groh-Wargo et al. (2) observe that preterm infants fed DHA and ARA have significantly more lean body mass at 12 months corrected age compared to infants fed formula without DHA and ARA. The infants fed DHA and ARA also have significantly lower fat mass compared to infants not fed DHA and ARA ($p<0.05$) (2). At 12 months CA, infants had greater lean body mass after receiving formula with DHA and ARA from

fungal oil and fish oil (6.83 kilograms [kg] ± 0.13) and DHA and ARA with egg-derived triglyceride and fish oil (7.00 kg ± 0.14) compared to infants who had consumed formula without DHA and ARA (6.53 kg ± 0.15). DHA and ARA supplemented infants also had lower fat mass when consuming formula with DHA and ARA from fungal oil and fish oil (2.60 kg ± 0.12) and DHA and ARA from egg-derived triglyceride and fish oil (2.60 kg ± 0.13) compared to infants who consumed the unsupplemented formula (3.07 kg ± 0.14), ($p<0.05$) (2).

Studies on the effects of formula supplemented with DHA on term infant growth are limited. In a study of term infants, Makrides et al. (7) fed infants formula supplemented with either DHA or DHA and ARA. No differences were found in weight or length between term infants fed formula with DHA, DHA and ARA, or without DHA or ARA. However, negative effects on infant length were found with maternal smoking, regardless of formula (7). Similarly, Auestad et al. (8) examined term infants and found no difference in weight or length among infants fed formula with DHA and ARA or without DHA and ARA. Male infants fed formula with DHA and ARA, however, gained more weight between enrollment and four months of age compared to infants receiving no DHA and ARA in the formula, but this difference in weight gain did not persist at 12 months (8). Two other studies by Auestad et al. (10, 11) also found no differences in weight or length of term infants through one year of age and through 39 months of age when formula was supplemented with DHA, DHA and ARA, or unsupplemented. In contrast to these findings, one study found term infants given formula supplemented with DHA and ALA had significantly lower body weight at four months of age ($p=0.02$) when high ALA (3.2%) was in the formula compared to child given the lowest amount of ALA

(0.4%). These infants had somewhat lower weight at eight months of age compared to three other formula groups with 0.4%, 1.0%, and 1.7% ALA, respectively, but the differences were not significant (9).

Effects of Maternal Smoking on Growth of Infants and Children

Length and height of infants and children is affected by maternal smoking during pregnancy. Mothers who smoked throughout gestation had children with lower length at birth and who were shorter in height through age eight (29). Koshy et al. (30) found children had lower average height-for-age z-scores when their non-smoking mothers were exposed to passive cigarette smoke during gestation and the father smoked compared to mothers not exposed to any smoke ($p<0.01$). If women were heavy smokers themselves, the difference was 2.76 times greater (30). Shorter stature in children has also been associated with paternal smoking ($p=0.009$). When both the mother and father were heavy smokers, children's chances of a shorter stature were increased 4.28 times ($p=0.012$) (30).

Maternal smoking during gestation also affects children's weight. The prevalence of being overweight was significantly higher in children born to mothers who smoked during gestation compared to those born to mothers who never smoked ($p<0.05$) (31). Koshy et al. (30) found a higher prevalence of obesity in male ($p=0.029$) and female ($p=0.014$) children born to mothers who smoked at any point during pregnancy compared to mothers who had not smoked. The same researchers reported the odds for risk of obesity increased by 1.61 ($p=0.002$) in children born to mothers who smoked (30). Holmo-Fasting et al. (32) determined the odds ratio for children to become overweight decreased from 2.83 (95% CI: 1.13, 7.10) to 1.29 (95% CI: 0.62, 2.67) when mothers

stopped smoking during gestation. In contrast, Chen and colleagues (29) report that prior to the age of eight, children had only moderately increased risks of becoming overweight from maternal smoking during gestation.

Timing of Maternal Smoking during Gestation on BMI

The timing and duration of maternal smoking during gestation may affect a child's weight later in life. Mendez et al. (31) found mothers who smoked only during the first trimester of gestation had the highest prevalence (39.7%) of overweight children. Maternal smoking in the first trimester doubled the odds of children between five and seven years of age becoming overweight (31). The researchers also found mothers who smoked only during the first trimester were more likely to have a child become obese later in life than mothers who continued smoking during the second and third trimesters. However, Chen et al. (29) found when mothers smoked during the third trimester of gestation, children had a greater risk of becoming overweight in the future compared to children of women who smoked only during the first trimester. Another study reported an increase in BMI and total fat mass in infants was associated with mothers who smoked during any point of gestation (33).

Children's BMI is affected when women smoke during pregnancy. A study by Holmo-Fasting and colleagues (32) found BMI was three percent lower in children born to mothers who never smoked compared to those who stopped smoking in early gestation. The researchers also found a 0.47 (95% CI: 0.10, 0.84) higher mean difference in BMI in children born to women who smoked and an adjusted odds ratio of 2.83 (95% CI: 1.13, 7.10) of becoming overweight by age four compared to those who did not (32).

In contrast, the same researchers reported that BMI decreased by four years of age in children born to smoking mothers when maternal smoking duration diminished in early gestation. At four years of age, BMI levels were similar for children of non-smoking mothers and those who stopped smoking early in gestation (BMI 15.81 kg/m^2 vs. 15.84 kg/m^2, respectively). These results suggest that BMI does not significantly differ between children born to mothers who never smoked and children of mothers who stopped smoking during pregnancy (32). Children of women who smoked throughout pregnancy, however, had a BMI of 16.09 kg/m^2 by age four. In contrast, Gilman et al. (34) did not find an association between children's BMI at seven years of age.

Catch-up Growth

The rate of weight gain in early life may affect weight later in life. Leary et al. (33) associated rapid weight gain early in life with future overweight status regardless of birth weight. Infants born to mothers who smoked experienced rapid catch-up growth during the first year after being born at low birth weights, and at age five, children had comparable or higher body weights than children born to non-smoking mothers. The rapid growth predicted increased risk of developing diabetes, hypertension, and cardiovascular disease as adults (29).

DHA Levels in Infants Born to Mothers Who Smoke

Maternal smoking during gestation may alter DHA levels and fat mass in infants. One study found lower DHA levels and lower DHA to ALA ratios in infants born to women who smoked during pregnancy (35). When mothers smoked only during the first trimester of gestation, infants had moderate levels of DHA and ALA. The same researchers suggested that some of the effects of smoking on growth might be due to a

limited placental transport of DHA to the fetus with maternal smoking. While this theory has not been tested, an increase in BMI and total fat mass in infants was associated with mothers who smoked any time during gestation (35).

Potential Mechanisms of Smoking Affecting Growth of Infants and Children

Fetal metabolism changes when women smoke during pregnancy. Children born to mothers who smoke during pregnancy may have growth restrictions and alterations in appetite and metabolism from nicotine and carbon monoxide exposure (29). Koshy et al. (30) indicate that paternal smoking can affect growth after birth and influence childhood appetite as well. Infants may adapt to smoking-related hypoxia and alter their appetite, similar to adjustments when people stop smoking and experience nicotine withdrawal (36, 37). Because nicotine suppresses appetite, infants feed more after birth compared to before birth due to less exposure to nicotine (38). Infants exposed to nicotine may also have an increased number of fat cell abnormalities related to changes in adipose tissue (30). Length and height differences in infants and children born to mothers who smoked during gestation may be the result of alterations in body fat, appetite control (39), and leptin and ghrelin responses (40, 41). However, Mendez et al. (31) claims there are no known mechanisms for how smoking during gestation affects weight gain and growth later in childhood.

Conclusion

The results of infant formula supplemented with DHA and ARA on growth of children zero to six years of age are inconsistent, although more suggest higher growth compared to unsupplemented children (2, 5, 6). Further research is warranted. Studies have indicated that children born to mothers who smoke during pregnancy are more

likely to have higher body weights and body mass indices with age (29-31, 33). The effect of maternal smoking status in conjunction with formula supplemented with DHA in relation to growth of children later in life has not been evaluated. Analyzing the effect of maternal smoking and formula supplemented with DHA could help to determine if either DHA intake or maternal smoking influences children's weight, height, and BMI.

Chapter 3

Methods

The DIAMOND (DHA Intake And Measurement Of Neural Development) study was a double-blinded, 2-phase, randomized, controlled, parallel-group, prospective trial to observe infants from birth to 18 months of age. The DIAMOND study was designed to determine the effects of infant formula supplemented with long-chain polyunsaturated fatty acids on visual and cognitive development in term infants. The primary objective of the study was to determine visual evoked potential acuity with secondary objectives that examined formula acceptance and tolerance, weight gain, length gain, head circumference gain, fatty acids and vitamin E, stereoacuity, cognitive development, and language development to 18 months of age. After 18 months, children were reenrolled and followed with similar assessment from age two to six years.

Ethics

The second phase of the study, from two to six years of age, was approved by the Institutional Review Board Ethics Committee and the Human Subjects Committee of the University of Kansas Medical Center in Kansas City, Kansas, as a project (HSC #10205) stemming from the parent trial (HSC #9198): The DIAMOND Study: A Double Masked, Randomized Controlled Clinical Trial of the Maturation of Infant Visual Acuity as a Function of the Dietary Level of Docosahexaenoic Acid. Written informed consent was obtained from each subject's parent(s) or guardian(s), and a copy of the signed written informed consent form was provided to them prior to participation in the clinical trial. Protected Health Information (PHI) was protected by having all information that could be linked to the subject in a locked file cabinet in a restricted access corridor of Smith West,

University of Kansas Medical Center. Only study personnel needing direct access to PHI were allowed access to collected data. All subject records were coded with initials and numbers. No information or data were stored on a laptop or on the internet to prevent identification of subjects. The study was unblinded to the study Principal Investigators (PIs) after all children reached 18 months of age, but personnel with access to subjects did not know their assignment until all children reached six years of age.

Research Setting and Subject Selection

The recruitment and enrollment of study subjects took place in prenatal clinics at two hospitals: Truman Medical Center (TMC) in Kansas City, Missouri, and the University of Kansas Medical Center (KUMC) in Kansas City, Kansas from September 2003 through September 2005. The study was conducted from September 2003 through October 2011.

To be included in the study, infants needed to be healthy, singleton-birth term infants 37 to 42 weeks gestation, weighing between 2490 and 4550 grams at birth, and formula-fed. Infants were excluded if they received human breast milk within 24 hours of randomization or had diseases or abnormalities that could affect growth, development, vision, or cognitive function; or who did not tolerate cow's milk infant formula or had poor intake of formula. Infants were also excluded if they were born to mothers with HIV, renal disease, hepatic disease, diabetes, substance abuse, or other chronic illnesses.

A total of 70 children were followed from birth to six years of age and were assessed at 6 weeks, 4 months, 6 months, 9 months, 12 months, 18 months, 2 years, 2.5 years, 3 years, 3.5 years, 4 years, 4.5 years, 5 years, 5.5 years, and 6 years. The first phase of the DIAMOND study enrolled 159 subjects followed from birth to 18 months of

age and were seen at 6 weeks, 4 months, 6 months, 9 months, 12 months, and 18 months. Children whose parents consented to the DIAMOND follow-up study (phase 2) were followed for growth and developmental outcomes to six years of age and were seen at 2 years, 2.5 years, 3 years, 3.5 years, 4 years, 4.5 years, 5 years, 5.5 years, and 6 years.

Infant Formula and Randomization

Infants enrolled in the study were assigned to one of four formulas. The formulas used in the study were cow's milk-based with the same nutrient levels, except the amount of DHA and ARA. The control group formula did not contain DHA or ARA (group 1). Formula group 2 had 0.32% fatty acids from DHA, containing 17 milligrams (mg) per 100 kilocalories (kcal), formula group 3 contained 0.64% DHA with 34 mg/100 kcal, and formula group 4 had 0.96% DHA with 51 mg/100 kcal. The DHA was from an algal source. All three DHA supplemented formulas contained 0.64% (34 mg/100 kcal) ARA from a fungal source.

Subjects were randomized by the study sponsor, Mead Johnson & Co., Evansville, Indiana, using a random-number generator function. Formula was packaged by the sponsor and only identified by color code. Each formula had two different color codes for a total of eight codes for study formulas. Groups were balanced for gender with each gender having an independent randomization. Envelopes prepared for each gender contained the code of the study formula to be assigned based upon the randomized lists. Following enrollment, the next sequential numbered envelope for a male or female was opened. Formula was provided for the infants at clinic visits, and study personnel were not aware of which formulation the infants received.

The formula was fed to infants until one year of age, and parents were encouraged to feed only formula for the first four months of age. After four months, infants could be fed additional foods as determined by physicians, but parents were told not to feed any DHA supplemented or enriched foods until one year of age.

Data Collection

Data were collected and entered into Microsoft Excel spreadsheets by trained research staff and registered dietitians. Anthropometric measures and dietary intakes were assessed at each clinic visit occurring at 6 weeks, 4 months, 6 months, 9 months, 12 months, 18 months, 2 years, 2.5 years, 3 years, 3.5 years, 4 years, 4.5 years, 5 years, 5.5 years, and 6 years. Formula intake data were collected at each clinic visit from six weeks through 12 months of age.

Demographic Data

Demographic data and maternal characteristics were collected through interviews and questionnaires. Information self-reported by mothers of infants enrolled in the study included maternal weight, height, education level, race and ethnicity, smoking status prior to and during pregnancy, number of packs of cigarettes smoked per day, and pack years of smoking, among other information. See appendix A for the demographic data collection forms.

Anthropometric Data

Birth weight, length, and head circumference were obtained within the first nine days of life from the participant's birth records. From six weeks through six years, weight was measured on a calibrated, standardized pediatric scale. A length board was

used to measure recumbent length, a stadiometer to measure height, and a flexible, non-stretchable vinyl measuring tape was used to measure head circumference.

Body weight was recorded to the nearest gram (for infants and children less than 18 months of age) or ounce (2 to 6 years of age) with infants only wearing a dry diaper and children wearing no shoes. Until children were two years of age, body length was measured once to the nearest tenth of a centimeter with the subject held in a recumbent position with one person holding the subject's head to contact with the fixed headboard and a second person holding the subject's knees flat and feet with the toes pointing directly upward, while moving the footboard firmly against the subject's heels. Height was measured once using a stadiometer at each visit between two and six years of age with the subject's feet flat on the floor against the wall, looking straight ahead, without shoes. Head circumference was measured to the nearest tenth of a centimeter using a flexible, non-stretchable vinyl measuring tape at the highest occipital circumference (18-21). See appendix B for the anthropometric data collection form.

All anthropometric measures from each clinic visit were used to calculate the Centers for Disease Control growth percentiles using Epi Info. software (18-21). The weight-for-length and length-for-age percentiles were calculated for subjects less than two years old, and stature-for-age and BMI-for-age percentiles were calculated for subjects between two and six years of age.

Weight, length/height, and head circumference measures were entered into the Epi Info. software program to obtain weight-for-age, length/stature-for-age, weight-for-length, and BMI-for-age percentiles. At age two, 54 of 79 total subjects were 23 months of age and not exactly 24 months of age by their estimated delivery date (EDD). To

accurately calculate the stature-for-age percentiles at age two, the 54 children were rounded to exactly 24 months of age in Epi Info. Height was not recorded as length and was not recumbent. Tests of growth percentiles with 24 months of age compared to 23 months were done by a t-test, and no significant difference was found (p=0.9). Except for stature-for-age, the exact age of the child was used and only the 24 month old children's data were adjusted. Weight-for-length percentiles were calculated for all ages, however if the child's height was greater than 121 centimeters (cm), the stature-for-age, height z-scores, weight-for-length percentiles, and weight-for-length z-scores were not able to be calculated. This resulted in the omission of weight-for-length percentiles and z-scores for one five year old child, four 5.5 year old children, and 11 six year old children.

For subjects missing one time point, the weight and length measurements were imputed. To impute data, a conversion factor was calculated from the average gain of all subjects before the missing time point and after the missing time point and then used to calculate the individual's data point. A total of 14 children were missing one or more data points. Of the 14, 11 were only missing one time point and data were imputed. Three children were missing more than one consecutive data point and were excluded from the analysis. CDC growth percentiles were determined using Epi Info. and the date of the measurement was the exact age of the child at the missing time point.

Formula Intake Data

Formula intake and tolerance were assessed at each clinic visit through 12 months of age by querying the subject's parent(s) or caregiver(s). We asked questions about the amount of formula consumed, consumption of other formulas or milk, and bowel

movements describing the infant's number, color, and consistency of stools for one day and any constipation, diarrhea, excess gas, or unusual fussiness. See appendix C for formula intolerance collection form.

Dietary Intake Data

Dietary intake was assessed using a 24-hour recall collected from the parent(s) or caregiver(s) by registered dietitians trained by Dr. Debra Sullivan's research team at the University of Kansas Medical Center at each clinic visit. Foods and beverages consumed the day before the clinic visit (i.e. 12 A.M. to midnight the day prior) were recorded and included times of eating, portion sizes, and brand names and ingredients used in preparation. Dietary intake was assessed using descriptions and tools of household measurements including measuring cups and spoons, pre-portioned bean bags of several amounts and sizes, labeled cups, and square, triangle, and circle shapes for sizes of foods. All tools had a reference chart to determine exact amounts. See appendix D for the 24-hour dietary recall collection form.

Dietary information from all clinic visits was entered into the Nutrition Data System for Research (NDS-R)® software program (v4.06_34 University of Minnesota, Minneapolis, Minnesota) and exported to NDS-R® version 2010 by a trained registered dietitian. Average caloric intake was calculated for each subject and each formula group at each study visit between six weeks and six years of age. Energy intake was entered and transferred into Microsoft Access and Microsoft Excel prior to analysis by IBM Statistical Package for the Social Sciences (SPSS)® 20. We excluded 24-hour dietary recalls that were missing one or more meals or with kilocalories (kcal) less than 40 kcal per kilogram (kcal/kg) or greater than 200 kcal/kg. Any recalls recorded as unreliable or

as non-typical were individually examined and were excluded with the same rules as stated above. See appendix E for a list of omitted 24-hour dietary recalls.

Training of Research Staff

The research staff and registered dietitians were trained to measure weight, length, height, and head circumference of infants and children by first obtaining measurements on staff to ensure reliability. Registered dietitians then measured children with supervision, and after further approval, they collected data on children individually. They were also trained in conducting 24-hour dietary recalls using the Multiple Pass Method by first interviewing staff to ensure reliability (42). The Multiple Pass Method used for 24-hour dietary recalls collected at the University of Kansas Medical Center consisted of first allowing the parent or guardian to list all foods and beverages eaten by the infant and child the day before the clinic visit with the interviewer using neutral probing questions. Second, the interviewer asked for details on all foods and beverages listed. Third, the interviewer reviewed the recall with the parent or guardian, probing for missing items (42). Research staff and registered dietitians were also trained to use Nutrition Data System for Research (NDS-R)® to enter recalls for nutritional analyses. Staff entering dietary recalls into NDS-R® were required to reliably enter 12 standardized 24-hour dietary recalls of hypothetical subjects ranging in age from six week to six years and were considered reliable if nutritional analyses were within five percent of nutrient levels when compared to master analyses.

Collection and Analysis of Data

Data collected prospectively for each subject included DHA (yes, no), DHA amount (0, 0.32, 0.64, 0.96% DHA), weight, length, and head circumference (absolute

and percentiles for age) and energy intake at each study visit. Maternal characteristics were determined by interviews and questionnaires. Maternal data included self-reported maternal weight at enrollment, last clinic visit, and at delivery; height; education level; race and ethnicity; smoking status prior to and during pregnancy; the number of packs of cigarettes smoked per day and the number of pack years of smoking. The data were entered into Microsoft Excel and transferred to IBM SPSS® Statistics 20 for statistical analyses. For demographic characteristics, means and standard deviations were calculated.

The effect of DHA supplementation in formula fed during the first year of life and BMI from two to six years of age and individual anthropometric parameters (weight, length, weight z-scores, length z-scores, and weight-for-length percentiles from birth to two years of age) were initially analyzed using repeated measures analysis of variance (ANOVA). Covariates included maternal smoking status and pack years smoked, maternal age, maternal education, and maternal BMI at enrollment. Results were considered significant if the p value was less than 0.05.

It became clear that this analysis was not the correct analysis, because lines were parallel and not intersecting. The repeated measures analysis dropped children who did not have all assessments as well. A second analysis was completed with a former collaborator of Dr. Susan Carlson, Dr. Betsy Tolley, using a Statistical Analysis System (SAS) procedure that created a trajectory for weight, height, and BMI percentiles for each child. The procedure allowed calculation of a median weight, height, BMI percentile, and energy intake for each group (DHA no, maternal smoking no; DHA yes, maternal smoking no; DHA no, maternal smoking yes; DHA yes, maternal smoking yes).

Additional variables tested were age (to determine if these variables were affected by increasing age between two and six years) and maternal BMI at the time the child's mother became pregnant. We did not test the effect of race because of the small number of degree of freedom (65).

Chapter 4

Results

The objectives of this thesis were to determine 1) if the concentration of DHA in formula consumed from birth to 12 months of age affects growth and particularly indicators of body fatness (weight-for-length and BMI-for-age) and 2) to determine if maternal smoking history affects these same indicators of growth and body fatness. The specific study questions were refined to focus on weight, height, and BMI percentiles from two to six years of age, but some data are included on weight and length between birth and two years of age. The CDC recommends using BMI-for-age percentiles after two years of age (18-21). It is not possible to calculate weight-for-length percentiles for all children to six years of age, because a large number of children exceed the length that can be used in this calculation (121 cm). Nevertheless, I included trajectories for absolute weight and length from birth to six years. These figures are useful to demonstrate that children who were fed formula with and without DHA did not differ in weight or length at birth, though children whose mothers smoked during pregnancy were lighter and shorter through four years of age (shown previously by another Master's of Science student, Nicole Kreber).

Subject Characteristics

Subject characteristics are shown in Table 1 for the original study population and the subset of subjects studied from birth through six years of age. Maternal characteristics are shown for the original study population and the subset of subjects though six years of age in Table 2, and Table 3 shows maternal and infant characteristics of the study population supplemented and unsupplemented with DHA and ARA. These

tables indicate the subset of subjects studied from birth to six years of age represents the original study population well, and results suggest the subgroup is similar to the original cohort except that more males than females were lost during the follow-up study.

Table 1. Infant characteristics of the original study subjects and the subset of study subjects.

Infants	**Original Study Population n=159**	**Subset Study Population n=70**
Male [n (%)]	75 (47.2%)	23 (32.9%)
Female [n (%)]	84 (52.8%)	47 (67.1%)
Weight at birth (g)*	3380 ± 414	3424 ± 360
Length at birth (cm)*	49.9 ± 2.09	50.01 ± 1.65
Head circumference at birth (cm)*	33.91 ± 2.66	34.20 ± 1.32

***** Reported as mean ± standard deviation (SD).

Table 2. Maternal characteristics of the original study subjects and the subset of study subjects.

Maternal Characteristics	**Original Study Population n=159**	**Subset Study Population n=70**
Weight at 1st clinic visit (lbs)*	165.06 ± 43.69	169.00 ± 43.23
Height (inches)*	64.39 ± 2.87	64.17 ± 3.14
BMI at 1st clinic visit (kg/m^2)*	27.90 ± 7.09	28.93 ± 7.06
Weight at last clinic visit (lbs)*	186.36 ± 44.07	195.74 ± 44.30
Weight at delivery (lbs)*	190.25 ± 42.25	197.07 ± 43.63
Age at delivery (years)*	23.91 ± 5.39	23.46 ± 4.28
Smoking before pregnancy [n (%)]	73 (45.9%)	33 (47.1%)
Smoking during pregnancy [n (%)]	49 (30.8%)	24 (34.3%)
Pack years smoked (packs per day x years smoked)*	1.72 ± 3.35	1.89 ± 3.41
Education obtained (years)*	12 ± 1.65	11.9 ± 1.47
Race		
White	59 (37.1%)	24 (34.3%)
Black/African American	98 (61.6%)	45 (64.3%)
Other	2 (1.26%)	1 (1.4%)

*Reported as mean ± standard deviation (SD).

Table 3. Maternal and infant characteristics of the subset study population supplemented and unsupplemented with DHA and ARA.

Infant Characteristics	Unsupplemented Study Population n=16	Supplemented Population n=54
Male [n (%)]	6 (37.5%)	17 (31.5%)
Female [n (%)]	10 (62.5%)	37 (68.5%)
Weight at birth (g)*	3354 ± 394	3424 ± 360
Length at birth (cm)*	49.6 ± 1.69	50.13 ± 1.64
Head circumference at birth (cm)*	34.26 ± 1.4	34.19 ± 1.31
Maternal Characteristics		
Weight at 1st clinic visit (lbs)*	168.99 ± 46.95	169.01 ± 42.60
Height (inches)*	63.93 ± 3.76	64.24 ± 2.97
BMI at 1st clinic visit (kg/m^2)*	29.12 ± 7.33	28.88 ± 7.05
Weight at last clinic visit (lbs)*	201.69 ± 53.06	193.76 ± 41.58
Weight at delivery (lbs)*	203.33 ± 52.92	194.78 ± 40.22
Age at delivery (years)*	22.75 ± 3.96	23.67 ± 4.39
Smoking before pregnancy [n (%)]	8 (50.0%)	25 (46.3%)
Smoking during pregnancy [n (%)]	4 (25.0%)	20 (37.0%)
Pack years smoked (packs per day x years smoked)*	1.52 ± 2.37	1.89 ± 3.41
Education obtained (years)*	12.0 ± 1.71	11.9 ± 1.41
Race [n(%)]		
White	2 (12.5%)	22 (40.7%)
Black/African American	14 (87.5%)	31 (57.4%)
Other	0	1 (1.9%)

*Reported as mean ± standard deviation (SD).

Effects of DHA and maternal smoking on BMI of children

Initial analyses were conducted using repeated measures analysis of variance (ANOVA). As noted previously, the n for these analyses is smaller than the available children in each group. All DHA supplemented groups (n=52; [0.32% DHA (n=15)], [0.64% DHA (n=16)], [0.96% DHA (n=23)]) were combined and compared to the control group without DHA supplementation (n=15). Covariates that were evaluated in the analyses were maternal BMI at enrollment, maternal education, and maternal smoking status and pack years [smoking (n=22) vs. non-smoking (n=45)].

A secondary analysis was conducted using Least Squares Means (LSM) and evaluated weight, length, BMI, and energy intake from two to six years of age. The covariates that were shown to be influential in the ANOVA were evaluated for significance (Table 4). Based on this analysis, maternal education and maternal BMI at enrollment, which trended toward significance, were not chosen for inclusion in the LSM analysis. DHA supplementation status, maternal smoking during pregnancy (yes or no), and child age were included in the LSM analysis.

Table 4. Evaluation of potential covariates in the analyses of the effect of DHA supplementation on growth of children.**

	Weight Percentile	**BMI**
DHA supplementation	0.0045*	0.4597
Child Age	0.5958	0.0363*
DHA supplementation	0.0070*	0.4136
Child Age	0.5950	0.0370*
Maternal education	0.2002	0.4320
DHA supplementation	0.0196*	0.4976
Child Age	0.6275	0.0444*
Maternal BMI at enrollment	0.9203	0.0700
DHA supplementation	0.0088*	0.4133
Child Age	0.5971	0.0350*
Smoking pack years	0.1375	0.5319
DHA supplementation	0.0056*	0.4103
Child Age	0.6001	0.0348*
Smoker	0.6517	0.4637
DHA supplementation	0.0322*	0.4439
Child Age	0.5593	0.0392*
Smoking during pregnancy (ppd)	<0.0001*	0.7657
DHA supplementation	0.0179*	0.519
Child Age	0.5265	0.0205*
Smoking during pregnancy	<0.0001*	0.1175
DHA supplementation * Smoking during pregnancy	0.3898	0.6227

*Indicates significance ($p \leq 0.05$).
**LSM.

Measures of BMI

The mean BMI and mean BMI percentiles were analyzed in DHA supplemented and unsupplemented children from two to six years of age. Results are illustrated in Figure 1 and Figure 2, respectively.

In children two to six years of age, maternal BMI at enrollment positively influenced the child's BMI ($p=0.0700$), though it did not quite reach significance. DHA supplementation resulted in an overall BMI from two to six years of age that was 0.23 units higher compared to formula without DHA and ARA, but the difference was not significant ($p=0.4976$). However, for every one year increase in age between two and six years of age, children's BMI increased by 0.20 ($p=0.0444$). Table 5 illustrates the effects of DHA supplementation on BMI of children through six years of age.

BMI-for-age percentiles of children from two to six years of age were not influenced by DHA supplementation ($p=0.1723$). Maternal smoking during pregnancy increased children's BMI percentiles by 23% compared to children of women who did not smoke during pregnancy ($p<0.0001$). There was no interaction between DHA supplementation and maternal smoking during pregnancy. Age positively influenced BMI percentiles as BMI percentiles increased by 6.5 percentiles every year from two to six years ($p<0.0001$). Table 6 illustrates the effects of all variables on BMI percentiles of children through six years of age.

We assessed how many children became overweight or obese according to BMI-for-age percentiles from two to six years of age. The American Academy of Pediatrics ask pediatricians to counsel families of children who become overweight (85-95th percentile of BMI-for-age percentiles) or obese (>95th percentile of BMI-for-age

percentiles) to encourage them to achieve a healthy weight by reducing energy intake and increasing energy expenditure (43). The CDC growth percentiles change, and after two years of age, weight-for-length percentiles cannot be calculated. As noted previously (13), the weight-for-length percentiles at two years of age are higher than the BMI percentiles. This is the most likely reason for the apparent reduction in overweight and obese children between 18 months and two years of age. We examined the number of children who were overweight and obese from two to six years of age in the cohort. This resulted in a high number of overweight and obese children starting at age four and continuing through age six. Results are illustrated in Tables 7.

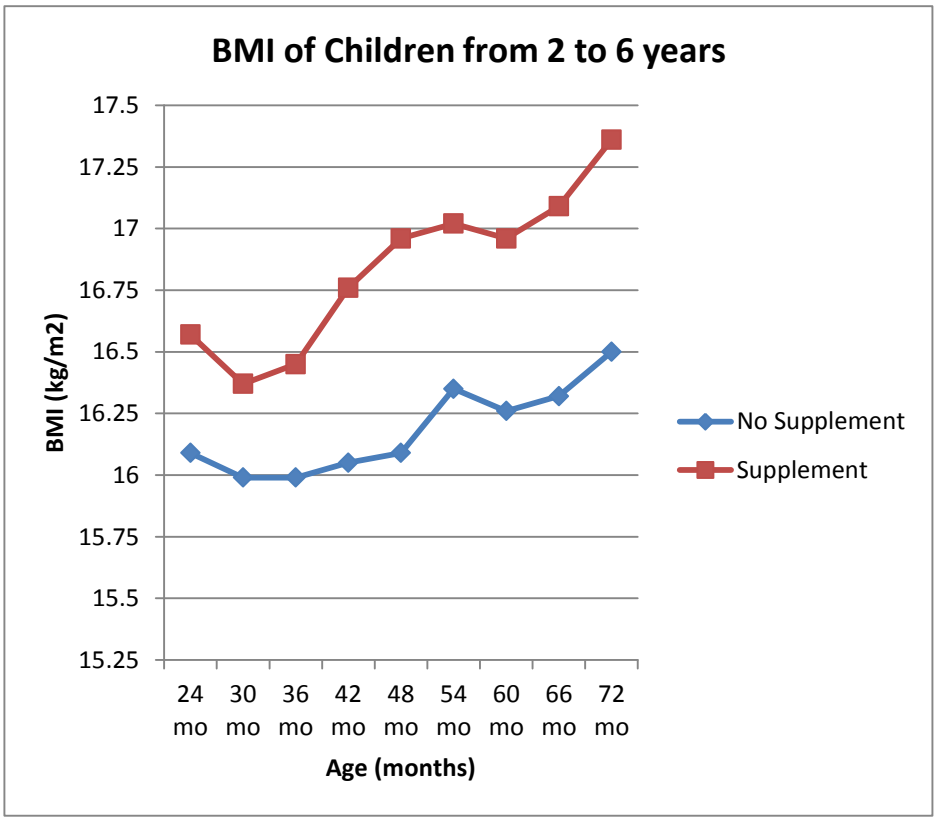

Figure 1. BMI from two to six years of age in DHA supplemented and unsupplemented children.

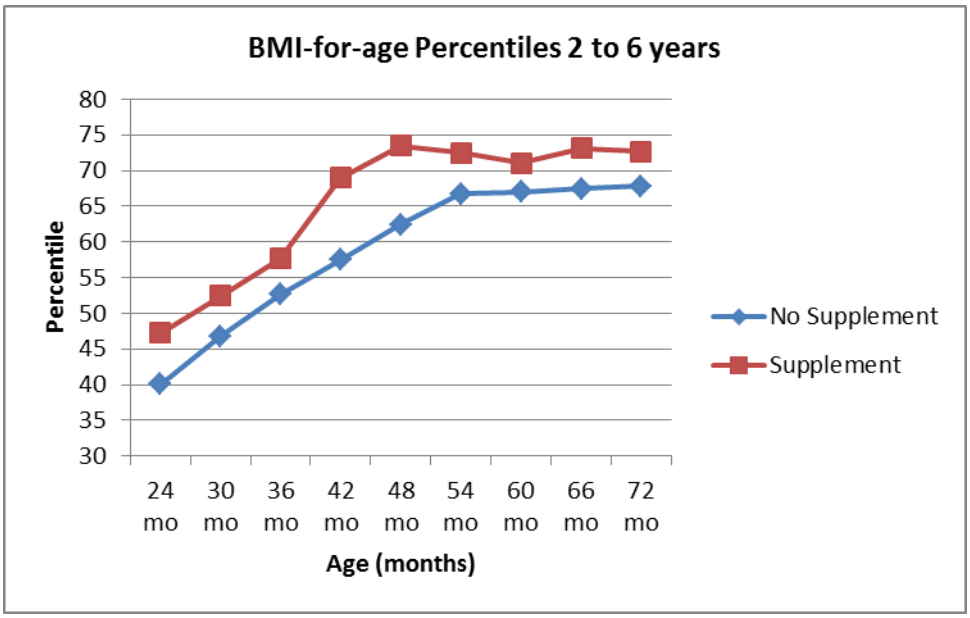

Figure 2. BMI-for-age percentiles from two to six years of age in supplemented and unsupplemented children.

Table 5. Effects of DHA supplementation, age, and maternal BMI on BMI of children through 6 years of age.*

	Estimate	Standard Error	Degrees Freedom	T Value	P Value
Intercept	16.9368	0.5617	64	30.15	<0.0001
DHA supplementation	0.2278	0.3340	64	0.68	0.4976
Age of child	0.1984	0.09680	66	2.05	0.0444
Maternal BMI at enrollment	0.03387	0.01865	460	1.82	0.0700

*LSM.

Table 6. Effects of DHA supplementation and maternal smoking on BMI percentiles of children through 6 years of age.*

	Estimate	Standard Error	Degrees Freedom	T Value	P Value
Intercept	58.6771	3.4017	65	17.25	<0.0001
DHA supplementation	14.5068	8.9185	65	1.63	0.1087
Age	6.4874	0.6523	68	9.94	<0.0001
Smoking (pregnancy)	30.5317	4.0729	65	7.50	<0.0001
DHA * Smoking (pregnancy)	14.9955	10.1573	65	1.48	0.1447

Final Model	Estimate	Standard Error	Degrees Freedom	P Value
DHA Supplementation	7.0091	5.0787	65	0.1723
Smoking (pregnancy)	23.0340	5.0787	65	<0.0001

*LSM.

Table 7. Overweight and obese children according to weight-for-length percentiles (birth to 2 years) and BMI-for-age percentiles (2 to 6 years) (n=70).

Age	85^{th} - 95^{th}%, n(%)	>95^{th}%, n(%)
Birth	3 (4%)	5 (7%)
6 weeks	8 (11%)	3 (4%)
4 months	9 (13%)	4 (6%)
6 months	6 (9%)	5 (7%)
9 months	12 (17%)	5 (7%)
12 months	11 (16%)	4 (6%)
18 months	10 (14%)	4 (6%)
2 year	4 (6%)	3 (4%)
2.5 year	10 (14%)	3 (4%)
3 year	5 (7%)	7 (10%)
3.5 year	12 (17%)	8 (11%)
4 year	18 (26%)	11 (16%)
4.5 year	12 (17%)	15 (21%)
5 year	11 (16%)	15 (21%)
5.5 year	17 (24%)	13 (19%)
6 year	11 (16%)	15 (21%)

Measures of Weight

The mean weights of subjects who consumed DHA supplemented formulas and the unsupplemented formula was compared from birth through six years. Weight was significantly higher from six weeks through six years of age in subjects who consumed the DHA supplemented formulas compared to those unsupplemented ($p=0.038$). Maternal education, a measure of socioeconomic status, was positively related to weight of children to six years of age ($p=0.006$), and maternal BMI was also positively related to weight of children ($p=0.007$). Results are illustrated in Figure 3.

DHA supplementation positively influenced weight z-scores from birth to six years of age ($p=0.048$) after correcting for covariates of maternal BMI at enrollment ($p=0.007$), maternal education ($p=0.043$), and maternal smoking pack years ($p=0.400$). Results are illustrated in Figure 4.

A second analysis that created a weight percentile trajectory for each child showed that DHA supplemented children had significantly higher weight percentiles than unsupplemented children ($p=0.0179$). Maternal smoking during pregnancy had an even more pronounced influence on weight percentiles with smoking related to a 32% increase in weight percentile ($p<0.0001$). When children born to mothers who smoked during pregnancy but who received DHA were compared to children whose mother did not smoke but who also received DHA, the weight percentiles were 91.9 compared to 53.9 mean percentile, respectively ($p<0.0001$). Likewise, among children who did not receive DHA, weight percentiles were higher in those whose mother's smoked during pregnancy compared to those whose mother's did not (69.7 and 43.4 mean percentiles, respectively, $p=0.0369$). The group of children with the lowest weight percentiles (43.4) was those

who did not receive DHA and had mothers who did not smoke. Table 8 illustrates the effects of DHA supplementation on weight percentiles of children through six years of age and includes the mean percentiles for the different DHA and smoking combinations.

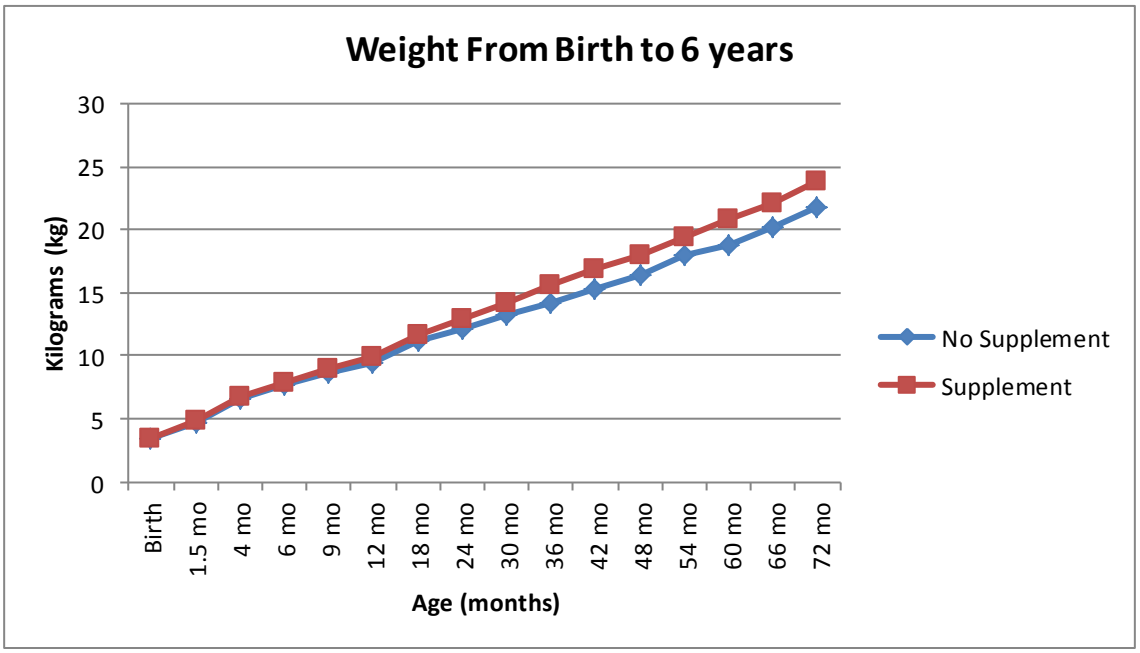

Figure 3. Weight (kg) from birth to six years in DHA supplemented and unsupplemented children. Independent effects of maternal education, maternal BMI at enrollment, and maternal smoking pack years were observed and adjusted for in the figure.

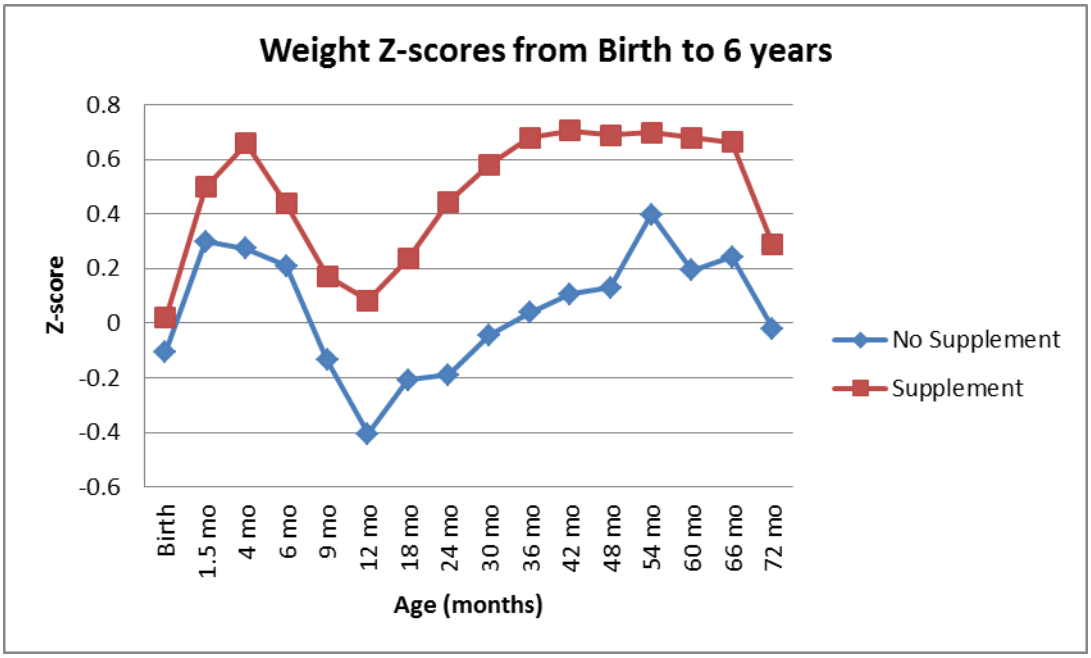

Figure 4. Weight z-scores from birth to six years in DHA supplemented and unsupplemented children.

Table 8. Effects of DHA supplementation on weight percentiles of children through 6 years of age.*

	Estimate	Standard Error	Degrees Freedom	F Value	P Value
Intercept	86.2924	4.3267	65	19.94	<0.0001
DHA Supplementation	22.2219	11.8531	65	1.87	0.0653
Age	1.4075	2.2108	68	0.64	0.5265
Smoking (pregnancy)	38.0094	5.4005	65	7.04	<0.0001
DHA * Smoking (pregnancy)	11.6790	13.4885	65	0.87	0.3898

Final Model	Estimate	Standard Error	T Value	P Value
DHA Supplementation	16.3824	6.7443	2.43	0.0179
Smoking (pregnancy)	32.1699	6.7443	4.77	<0.0001

Effect	Estimate	Standard Error	Degrees Freedom
No DHA supplementation	56.5410	10.3084	65
DHA supplementation	72.9234	8.6806	65
No smoking (pregnancy)	48.6473	8.8553	65
Smoking (pregnancy)	80.8172	10.1587	65
No DHA * No smoking (pregnancy)	43.3759	9.9344	65
No DHA * Smoking (pregnancy)	69.7062	13.7920	65
DHA * No smoking (pregnancy)	53.9187	8.8805	65
DHA * Smoking (pregnancy)	91.9281	9.2965	65

*LSM.

Measures of Length/Height

When analyzing the mean length/height of children, DHA supplementation did not appear to influence height to six years of age (p=0.137). However, maternal education was positively related to height from four months through six years (p=0.024). Results are illustrated in Figure 5. DHA supplementation did not influence length z-scores from birth to six years of age (p=0.096), nor did maternal BMI at enrollment (p=0.054), maternal education (p=0.166), or maternal pack years smoked (p=0.662). Results are illustrated in Figure 6.

A second analysis that calculated a height trajectory from two to six years of age for each child demonstrated that children supplemented with DHA were significantly taller than unsupplemented children (p=0.0039). Children of women who smoked during pregnancy were 17.6% taller than children born to nonsmoking mothers (p=0.0006). No interaction was found between DHA and maternal smoking during pregnancy (p=0.3745). Results are shown in Table 9. Table 10 summarizes the least squares mean weight, height, and BMI percentiles for children two to six years of age by DHA and maternal smoking.

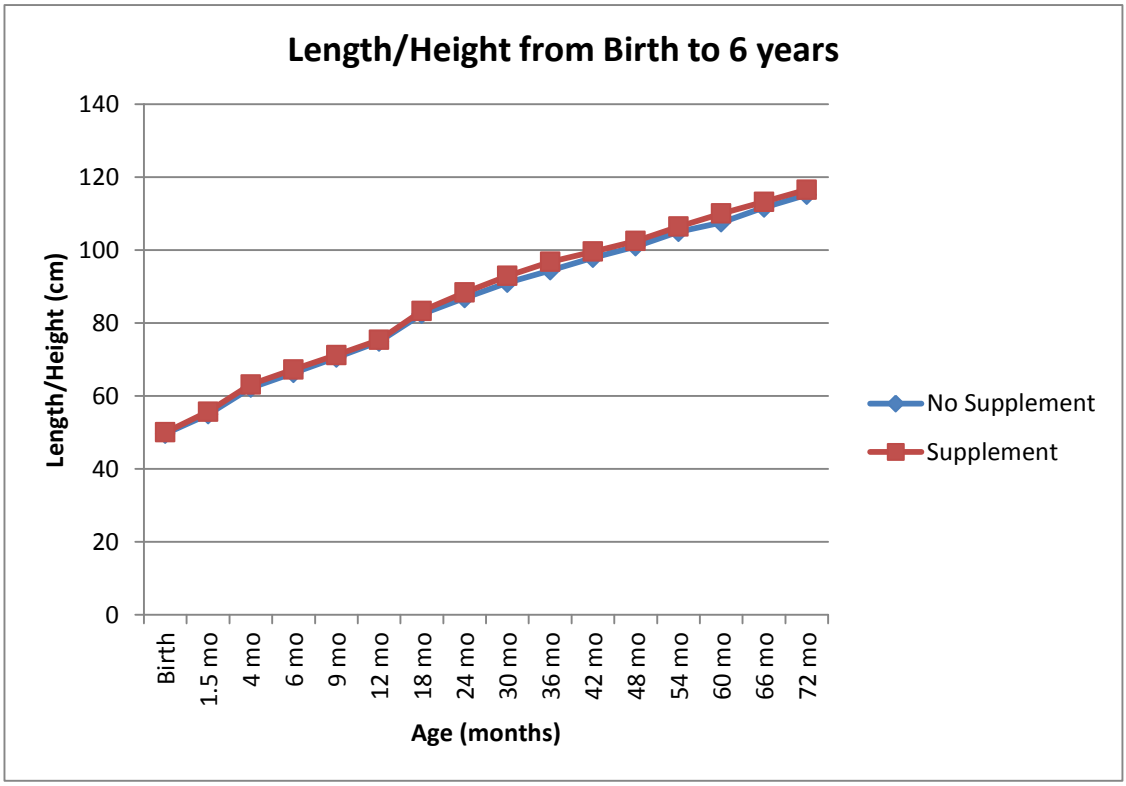

Figure 5. Length/Height (cm) from birth to six years in DHA supplemented and unsupplemented children. Independent effects of maternal education was observed and adjusted for in the figure.

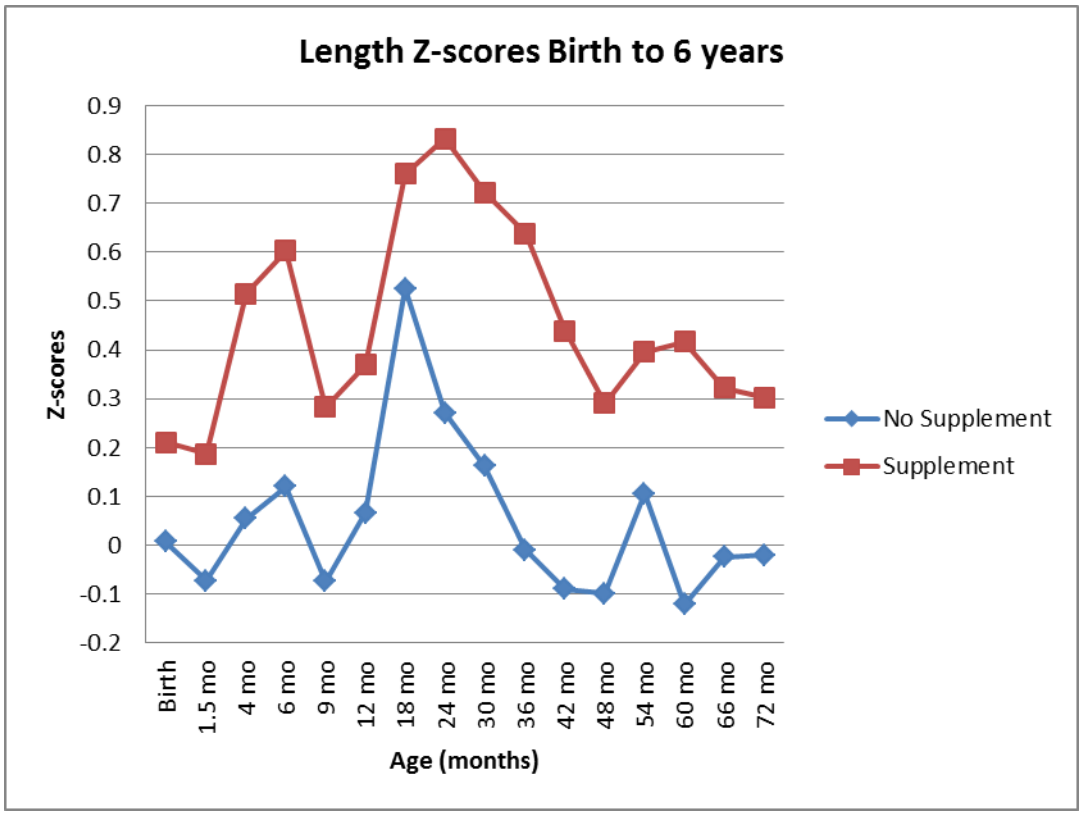

Figure 6. Length/Height (cm) z-scores from birth to six years in DHA supplemented and unsupplemented children.

Table 9. Effects of DHA supplementation on height percentiles of children through 6 years of age.*

	Estimate	Standard Error	Degrees Freedom	T Value	P Value
Intercept	92.0199	3.1379	65	29.33	<0.0001
DHA Supplementation	18.9377	8.5536	65	2.21	0.0303
Age of child	-3.5036	0.6694	68	-5.23	<0.0001
Smoking (pregnancy)	21.9615	3.9092	65	5.62	<0.0001

Final Model	Estimate	Standard Error	T Value	P Value
DHA Supplementation	14.5810	4.8720	2.99	0.0039
Smoking (pregnancy)	17.6048	4.8720	3.61	0.0006

*LSM.

Table 10. Effects of DHA and smoking during pregnancy on weight, height, and BMI percentiles for children two to six years of age.*

Percentile	Estimate	Standard Error
BMI Percentiles		
DHA supplementation	7.0091	5.0787
Smoking (pregnancy)	23.0340	5.0787
No DHA * No smoking (pregnancy)	54.6048	4.4560
No DHA * Smoking (pregnancy)	70.1410	8.4572
DHA * No smoking (pregnancy)	54.1161	2.9215
DHA * Smoking (pregnancy)	84.6478	3.5823
Weight Percentiles		
DHA supplementation	16.3824	6.7443
Smoking (pregnancy)	32.1699	6.7443
No DHA * No smoking (pregnancy)	43.3759	9.9344
No DHA * Smoking (pregnancy)	69.7062	13.7920
DHA * No smoking (pregnancy)	53.9187	8.8805
DHA * Smoking (pregnancy)	91.9281	9.2965
Height Percentiles		
DHA supplementation	14.5810	4.8720
Smoking (pregnancy)	17.6048	4.8720
No DHA * No smoking (pregnancy)	45.8080	4.5738
No DHA * Smoking (pregnancy)	59.0562	8.2713
DHA * No smoking (pregnancy)	56.0324	3.2375
DHA * Smoking (pregnancy)	77.9939	3.8004

*LSM.

Energy Intake

Average energy intakes (kcal) from 24-hour dietary recalls were generated for each age and formula group (Table 11). An analysis of energy intake from two to six years found no effect of DHA supplementation (p=0.7081), maternal smoking during pregnancy (p=0.8603), or age (p=0.3784). Results are illustrated in Table 12.

Table 11. Mean total kilocalories and kcal/kg consumed for all children and by formula group.*

	6 wk	4 mo	6 mo	9 mo	12 mo	18 mo	2 yr	2.5 yr	3 yr	3.5 yr	4 yr	4.5 yr	5 yr	5.5 yr	6 yr
Energy intake (all)	620	703	816	937	1101	1362	1473	1517	1559	1600	1581	1593	1671	1403	1378
Energy intake/kg (all)	126	105	104	104	114	117	118	109	106	100	98	90	91	84	74
0.00% DHA	128	112	106	113	128	124	128	113	108	104	107	91	103	89	70
0.32% DHA	131	109	104	98	105	124	116	116	116	99	101	86	75	78	70
0.64% DHA	120	101	104	106	120	110	115	108	102	93	91	82	96	82	84
0.96% DHA	123	96	103	99	104	108	112	99	96	104	92	99	91	87	70

*Reported as mean; ANOVA.

Table 12. Influence of DHA and smoking during pregnancy on energy intake in children from two to six years of age.*

	Estimate	Standard Error	Degrees Freedom	T Value	P Value
DHA Supplementation	-38.9574	103.59	65	-0.38	0.7081
Smoking (pregnancy)	18.2988	103.59	65	-0.18	0.8603
Age	-14.6563	16.5315	65	-0.89	0.3784

Energy Intake	Estimate	Standard Error
DHA supplementation	1512.25	43.5024
Smoking (pregnancy)	1540.88	91.8023
No DHA * No smoking (pregnancy)	1530.79	86.3579
No DHA * Smoking (pregnancy)	1571.62	169.92
DHA * No smoking (pregnancy)	1514.36	52.5392
DHA * Smoking (pregnancy)	1510.13	67.0465

*LSM.

Chapter 5

Discussion

This study finds higher weight and height but not higher BMI of children supplemented with DHA, which contrast with previously reported findings that no differences exist in growth of term infants fed DHA supplemented formula (7-12). One study did find a higher BMI ($p<0.022$) among children at 2.5 years of age whose mothers were given a fish oil supplement compared to mothers given an olive oil supplement during lactation (0 to 4 months of lactation), but no differences were found in weight, length, or head circumference through 9 months of age (44). Another study found infants on a fish oil supplement from nine to 18 months of age had lower skinfold ratios at 18 months than infants fed a sunflower oil supplement ($p=0.02$) (45).

Previous research has not analyzed the growth of term infants fed DHA supplemented formulas through six years of age, rather only through 39 months (11). The length of time infants were fed DHA supplemented formula differed among studies, ranging from four months to one year of age (7-12). The source of DHA and ARA also varied among previous studies (egg yolk, fish oil, soy oil, coconut oil, or high oleic sunflower oil). In addition, other studies have compared formulas with different percentages of DHA, DHA and ARA, and no DHA and ARA (7-12). We fed three different concentrations of DHA with a fixed amount of ARA in formula to term infants, which contained higher DHA and ARA concentrations, excluding the control formula, than earlier studies had used. Because there was no effect of DHA and ARA dose on child growth, the analysis collapsed all children who received DHA and ARA supplementation and compared them to children who did not receive any

supplementation (13). These may be contributing factors as to why our results contrast with existing research. Another factor may be that differences in weight and length appear to emerge around two years of age. By following children from two to six years, when BMI increases progressively, we may have been able to better identify effects of both smoking and DHA and ARA supplementation. Finally, United States children may be more overweight and obese than in other countries, which could allow environmental effects to emerge.

An examination of the effects of maternal smoking status clearly shows that maternal smoking during pregnancy is related to higher child weight and length and also higher BMI, which implies increased body fatness. In contrast to DHA, which increased both weight and length but not BMI percentiles, the increase in weight percentiles with maternal smoking was greater than the increase in length percentiles. It is this difference that resulted in a significant increase in BMI for children of women who smoked. An earlier examination of growth in this cohort showed that children of smoking women were shorter and lighter at birth and more likely to be overweight and obese by four years of age (13). Another study found that maternal smoking had a negative effect on length when term infants were fed DHA and ARA supplemented formulas for one year, suggesting maternal smoking has a more influential role in growth than DHA and ARA supplementation (7). The impact of maternal smoking status on children has been shown to result in shorter infants at birth and shorter children to age eight, which contrasts with our findings but are consistent with previous reports from several studies that indicate maternal smoking during gestation increases a child's risk of becoming overweight or obese later in life (29-31, 33).

A high percentage of women enrolled in the study had smoked before pregnancy [n=73 (45.9%)] and/or smoked during pregnancy [n=49 (30.8%)]. In the subset population studied, the percentage of women who smoked before and/or during pregnancy remained high [smoked before pregnancy, n=33 (47.1%) and smoked during pregnancy, n=24 (34.3%)], which provided a large enough number to reasonably evaluate the effect of smoking during pregnancy.

Our data for overweight and obesity indicate slightly higher incidences than the CDC's Pediatric Nutrition Surveillance System (PedNSS) 2008 report, which shows a 14.6% prevalence of childhood obesity in low-income, preschool aged children through four years of age (46). By age four, 23.4% of the children in our cohort were overweight and 15.6% were obese.

From the analysis of energy consumed by each formula group by gender, no significance resulted at any age. Therefore, gender was not a factor in energy consumed; Maternal education, maternal BMI, and DHA supplementation in formula consumed for one year of life positively influence weight of children through six years of age but not energy intake. The trend toward higher child BMI is similar to previous findings that mothers who are overweight are more likely to have overweight children (17).

Limitations

A limitation of this study was the small number of subjects in the unsupplemented DHA group compared to the subjects fed one of three DHA supplemented formulas (n=15 and 54, respectively). Because there was no effect of DHA and ARA dose on child growth, all DHA supplemented groups were combined (13). The combination of DHA supplemented children compared to unsupplemented children resulted in an uneven

subject number. This could have resulted in a Type 2 error, in particular finding that supplemented children did not have higher BMI-for-age percentiles than those who were not supplemented, when in fact a larger group may have shown an effect on BMI-for-age percentiles. The fact that DHA supplementation increased weight and length percentiles, despite the low number of control, suggests that the effect size on growth is quite large, although a Type 1 error cannot be ruled out.

Another limitation was the disproportional distribution of race in the control and supplemented groups. The DHA supplemented children were similarly represented by white and black/African American races, but the distribution of race in unsupplemented children included more black/African American subjects. There were also more female than male children who completed the study, which could have influenced absolute growth. However, the CDC percentiles for weight, height, and BMI that served as a basis for the main study questions do account for gender differences.

The 24-hour dietary recalls and questions concerning formula intake and tolerance are self-reported and subjective, allowing for error. Over or under estimation of individual dietary intake may have occurred by the caregiver(s) or guardian(s) due to distortions in portion sizes eaten by infants, toddlers, and young children and due to children spending time outside of a parent's care. In addition, growth and development is not only related to DHA supplementation and maternal smoking status, but it is also related to dietary factors including the type and amount of formula consumed, type and time point of introduction of solid foods to infants and toddlers, and quality of the diet. It is also influenced by genetics.

The weight-for-length percentiles cannot reliably be used after age four because children who achieve a length greater than 121 centimeters are omitted. This is one of the reasons we chose BMI-for-age percentiles to represent body fatness from two to six years of age. The weight-for-length percentiles do not illustrate groups reliably until four years.

Future Studies

It has been established that preterm infants fed a formula supplemented with DHA improves growth of these infants (2, 5, 6). Of the published studies on the growth of term infants fed DHA supplemented formula, none find any differences in weight or length (7-12). However, when DHA is supplemented to the mother during lactation, a child's BMI increases, and if fish oil is supplemented to the infant, lower skinfold ratios result (44, 45). Further analyzing children through school age, specifically from two to six years of age could help identify the environmental conditions that contribute to a yearly increase in BMI and that could contribute to their risk of becoming overweight or obese. Identifying these risks could also lead to interventions.

Although we looked only at energy intake, it is feasible that the diet of children of women who smoke during pregnancy differs in quality. This could contribute to differences in body composition, particularly to increased body fatness. It would be worthwhile to evaluate the diet quality of children in this study by maternal smoking during pregnancy as there may be differences in childhood feeding practices between smokers and nonsmokers, further programming a child's growth. If this is not the case, one might more strongly consider some physiological, endocrine, or motivational changes caused by maternal smoking exposure during fetal life.

Studies to evaluate body composition using more sophisticated analyses (e.g., assessment of body composition by air displacement plethysmography (ADP)) could be valuable additions to the literature related to maternal smoking.

Chapter 6

Summary

Docosahexaenoic acid (DHA) is a long chain omega-3 polyunsaturated fatty acid essential for visual, cognitive, and central nervous system development in infants (1, 2). DHA was added to infant formulas in the United States in 2002 (3). Studies have investigated the effects of formula supplemented with DHA on weight and body mass index (BMI) of children, but results are mixed. Three studies indicate DHA-supplemented formula fed to preterm infants improves weight gain (2, 5, 6). Six studies report the effects of DHA-supplemented formula fed to term infants and find no differences in weight or length (7-12).

This study was a double-blind, 2-phase, randomized, controlled, parallel-group, prospective trial. The primary outcome of the study was to determine the effects of DHA and ARA supplementation in formula on visual evoked potential acuity of infants. For the purposes of this thesis project, the outcomes were to determine 1) if the concentration of DHA in formula (0, 0.32%, 0.64%, 0.96% of the total fatty acids from DHA) consumed from birth to 12 months of age affects growth (BMI-for-age percentiles) and 2) to determine if maternal smoking history affects the same indicator of growth and body fatness of children through six years. All DHA supplemented groups (n=52; 0.32%, 0.64%, 0.96% DHA) were combined and compared to the control group with no DHA supplementation after group differences among the DHA groups were not found.

Between two and six years of age, DHA supplementation increased both weight-for-age and height-for-age percentiles in children but not BMI-for-age percentiles, whereas maternal smoking during pregnancy increased weight, height, and BMI

percentiles. DHA supplementation resulted in an overall BMI that was 0.23 units higher compared to formula without DHA and ARA, and maternal BMI at enrollment positively influenced the child's BMI, though neither reached significance. However, maternal smoking during pregnancy significantly increased children's BMI percentiles by 23% compared to children of women who did not smoke during pregnancy. There was no interaction between DHA supplementation and maternal smoking during pregnancy on children's BMI-for-age percentiles.

Maternal smoking during pregnancy had an even more pronounced influence on weight percentiles with smoking related to a 38% increase in weight percentile ($p<0.0001$). When children born to mothers who smoked during pregnancy but who received DHA were compared to children whose mother did not smoke but who also received DHA, the weight percentiles were significantly higher. The group of children with the lowest weight percentiles was those who did not receive DHA and had mothers who did not smoke. Children supplemented with DHA were significantly taller than unsupplemented children by 19 percentiles, but children of women who smoked during pregnancy were also significantly taller (22 percentiles) than children born to nonsmoking mothers. Energy intake was not related to either DHA intake or maternal smoking during pregnancy, suggesting the effects were unrelated to differences in energy intake.

Further research is warranted to determine the most beneficial amount of DHA supplemented in formula fed to term infants from birth through 12 months of age and the effects on the long term growth of children. Additional research is needed to determine a more specific critical time point in which children may become overweight or obese later

in life, as well as the body composition of children in relation to DHA and maternal smoking.

Chapter 7

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Appendix A

Subjects Demographic Data Collection Form

INVESTIGATOR	PROTOCOL	RANDOM CODE	DATE
CARLSON	HSC #10205		

DEMOGRAPHICS

Maternal Education

Paternal Education

Does anyone living in the child's home smoke?

☐ No ☐ Yes If yes, how many people smoke & how many ppd? _____

List any maternal allergies:

_____

Including the child enrolled in this study, how many children 13 years of age or younger live in your house?

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 or more

Do any pets live in the child's home?

☐ No ☐ Yes If yes, how many pets? _____
What kind?
_____

Do you take your child to a daycare (facility or homecare) with other infants and children?

☐ No

☐ Yes, with 1 to 5 children

☐ Yes, with 6 to 10 children

☐ Yes, with more than 10 children

Appendix B

Anthropometric Data Collection Form

INVESTIGATOR	PROTOCOL	INITIALS	RANDOM CODE	DOB
CARLSON	HSC #10205			

ANTHROPOMETRICS

2 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm
2.5 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm
3 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm
3.5 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm
4 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm

INVESTIGATOR	PROTOCOL	INITIALS	RANDOM CODE	DOB
CARLSON	HSC #10205			

ANTHROPOMETRICS PAGE 2

4.5 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm
5 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm
5.5 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm
6 Year Visit	Weight		g
MO DA YEAR	Length		cm
	Head circumference		cm

Appendix C

Formula Tolerance Collection Form

Mead Johnson Nutritionals

SOURCE DOCUMENTS CHECKED
Monitor's Initials _____ Date _____

INVESTIGATOR	PROJECT	STUDY	SUBJECT/RANDOM CODE	FORM ID
CARLSON	3370-4	1018	_____ - _____	D

SUBJECT'S INITIALS (F M L) | SUBJECT'S BIRTHDATE (MO DA YEAR)

STUDY VISIT (Check One):

☐ VISIT 3 (Age: 42 days* ±7) ☐ VISIT 4 (Age: 120 days* ±7) ☐ VISIT 5 (Age: 180 days* ±7) ☐ VISIT 6 (Age: 275 days* ±7) ☐ VISIT 8 (Age: 365 days* ±7)

* age adjusted based on Estimated Date of Confinement (EDC)

DIET AND TOLERANCE RECALL

Visit Date: (MO DA YEAR)

DIET RECALL

1. How much study formula has your baby drank in the past 24 hours? _____ oz
2. Are you still feeding your baby the study formula?

☐ Yes ☐ No *If No, when did you stop: (MO DA YEAR) Why? _____

**If more than 5 consecutive days, infant must be dropped from study.*

3. Since the last study visit, has your baby consumed any of the following milks, cereals, or foods:

Milk	☐ Yes ☐ No	Poultry	☐ Yes ☐ No		
Cereal	☐ Yes ☐ No	Fish	☐ Yes ☐ No		
Vegetables	☐ Yes ☐ No	Eggs	☐ Yes ☐ No		
Fruits	☐ Yes ☐ No	Cheese	☐ Yes ☐ No		
Juices	☐ Yes ☐ No	Cottage cheese	☐ Yes ☐ No		
Red meats	☐ Yes ☐ No	DHA supplemented foods	☐ Yes ☐ No	If YES, name:	_____

TOLERANCE RECALL

1. How many bowel movements did your baby have in the past 24 hours? _____
2. What was the general consistency of your baby's bowel movements over the past 24 hours? (Check ONLY one)

No Bowel Movement - 0	Hard - 1	Formed - 2	Soft - 3	Loose - 4	Watery - 5
☐	☐	☐	☐	☐	☐

1 = Dry, hard pellets
2 = Definite shape, not dry
3 = No definite shape, pasty
4 = No shape, some water
5 = No shape, mainly water

3. What was the general color of the bowel movements over the past 24 hours? (Check ONLY one)

No Bowel Movement - 0	Brown - 1	Green - 2	Yellow - 3	Black - 4
☐	☐	☐	☐	☐

4. Has your baby been unusually fussy in the past 24 hours? (check one): ☐ Yes* ☐ No
5. Has your baby had excessive problems with gas in the past 24 hours? (check one): ☐ Yes* ☐ No
6. Has your baby had diarrhea in the past 24 hours? (check one): ☐ Yes* ☐ No
7. Has your baby been constipated in the past 24 hours? (check one): ☐ Yes* ☐ No

***NOTE:** Any "Yes" response to questions 4-7 should result in the entry of an Adverse Event. Ask parents during visit if infant has been sick with any other problems, and if so, record as adverse event.

2-07-03
3370-4.D

Appendix D

24-Hour Dietary Recall Collection Form

24-Hour Dietary Recall Form

Visit:_____

Random #_____ Date of Intake:_____ DOB:_____ EDC:_____

Time	Food/Beverage	Ingredients/Preparation	Amount

Intake: Typical More than Usual Less than Usual Why? _____

_____

Recall: Reliable Unable to recall meals? Unreliable for other reasons? Why? _____

_____

Vitamin/Mineral/Supplement Use? _____

Home / Daycare / Babysitter Number of people responsible for feeding _____

Interviewer Initials: _____

Appendix E

List of Omitted 24-Hour Dietary Recalls

Unable to recall one or more meals: 95 total
Subject 139 at 4 months

Subject 87 at 6 months

Subject 80 at 9 months

Subject 74 at 12 months
Subject 124 at 12 months

Subject 2 at 18 months
Subject 46 at 18 months
Subject 60 at 18 months
Subject 84 at 18 months
Subject 89 at 18 months
Subject 91 at 18 months
Subject 125 at 18 months

Subject 5 at 2 years
Subject 24 at 2 years
Subject 35 at 2 years
Subject 43 at 2 years
Subject 62 at 2 years
Subject 102 at 2 years
Subject 103 at 2 years

Subject 9 at 2.5 years
Subject 139 at 2.5 years

Subject 103 at 3 years

Subject 46 at 3.5 years
Subject 108 at 3.5 years
Subject 127 at 3.5 years

Subject 97 at 4 years
Subject 102 at 4 years
Subject 135 at 4 years
Subject 148 at 4 years
Subject 154 at 4 years

Subject 32 at 4.5 years
Subject 48 at 4.5 years
Subject 102 at 4.5 years
Subject 110 at 4.5 years
Subject 112 at 4.5 years
Subject 120 at 4.5 years
Subject 131 at 4.5 years

Subject 35 at 5 years
Subject 48 at 5 years
Subject 75 at 5 years
Subject 77 at 5 years
Subject 88 at 5 years
Subject 100 at 5 years
Subject 112 at 5 years
Subject 113 at 5 years
Subject 120 at 5 years
Subject 124 at 5 years
Subject 131 at 5 years
Subject 134 at 5 years

Subject 11 at 5.5 years
Subject 17 at 5.5 years
Subject 24 at 5.5 years
Subject 35 at 5.5 years
Subject 39 at 5.5 years
Subject 48 at 5.5 years
Subject 60 at 5.5 years
Subject 61 at 5.5 years
Subject 74 at 5.5 years
Subject 77 at 5.5 years
Subject 80 at 5.5 years
Subject 84 at 5.5 years
Subject 113 at 5.5 years
Subject 134 at 5.5 years
Subject 139 at 5.5 years
Subject 147 at 5.5 years
Subject 149 at 5.5 years
Subject 154 at 5.5 years
Subject 155 at 5.5 years
Subject 159 at 5.5 years

Subject 5 at 6 years
Subject 11 at 6 years
Subject 13 at 6 years
Subject 24 at 6 years
Subject 30 at 6 years
Subject 32 at 6 years
Subject 39 at 6 years
Subject 48 at 6 years
Subject 60 at 6 years
Subject 61 at 6 years
Subject 77 at 6 years
Subject 84 at 6 years
Subject 87 at 6 years

Subject 96 at 6 years
Subject 100 at 6 years
Subject 102 at 6 years
Subject 108 at 6 years
Subject 113 at 6 years
Subject 124 at 6 years
Subject 126 at 6 years
Subject 127 at 6 years
Subject 134 at 6 years
Subject 135 at 6 years
Subject 154 at 6 years
Subject 155 at 6 years
Subject 158 at 6 years

Over reporting (>200 kcals/kg): 23 total
Subject 9 at 6 weeks
Subject 85 at 6 weeks
Subject 122 at 6 weeks
Subject 125 at 6 weeks
Subject 140 at 6 weeks

Subject 50 at 6 months
Subject 149 at 6 months

Subject 17 at 9 months
Subject 101 at 9 months

Subject 59 at 12 months
Subject 95 at 12 months

Subject 4 at 18 months
Subject 19 at 18 months
Subject 98 at 18 months
Subject 120 at 18 months
Subject 121 at 18 months
Subject 147 at 18 months

Subject 88 at 2 years
Subject 120 at 2 years
Subject 143 at 2 years
Subject 147 at 2 years

Subject 97 at 2.5 years
Subject 108 at 2.5 years

Underreporting (<40 kcal/kg): 54 total
Subject 118 at 6 months

Subject 54 at 9 months

Subject 42 at 12 months
Subject 118 at 12 months

Subject 87 at 18 months
Subject 89 at 18 months

Subject 35 at 2 years

Subject 9 at 4 years
Subject 46 at 4 years
Subject 97 at 4 years

Subject 9 at 4.5 years
Subject 32 at 4.5 years
Subject 48 at 4.5 years
Subject 62 at 4.5 years
Subject 110 at 4.5 years
Subject 112 at 4.5 years

Subject 35 at 5 years
Subject 77 at 5 years
Subject 97 at 5 years
Subject 112 at 5 years
Subject 134 at 5 years

Subject 5 at 5.5 years
Subject 9 at 5.5 years
Subject 11 at 5.5 years
Subject 17 at 5.5 years
Subject 35 at 5.5 years
Subject 39 at 5.5 years
Subject 46 at 5.5 years
Subject 48 at 5.5 years
Subject 63 at 5.5 years
Subject 84 at 5.5 years
Subject 97 at 5.5 years
Subject 113 at 5.5 years
Subject 131 at 5.5 years
Subject 139 at 5.5 years
Subject 149 at 5.5 years
Subject 155 at 5.5 years

Subject 159 at 5.5 years

Subject 5 at 6 years
Subject 9 at 6 years
Subject 48 at 6 years
Subject 61 at 6 years
Subject 62 at 6 years
Subject 77 at 6 years
Subject 84 at 6 years
Subject 88 at 6 years
Subject 97 at 6 years
Subject 102 at 6 years
Subject 109 at 6 years
Subject 127 at 6 years
Subject 139 at 6 years
Subject 147 at 6 years
Subject 154 at 6 years
Subject 155 at 6 years

Appendix F

Parent Study Consent Form: Birth to 18 Months of Age

CONSENT FORM

The Effects of Infant Formula Supplemented with Long Chain Polyunsaturated Fatty Acids on Visual Development in Term Infants

Protocol #3370-4
Sponsor: Mead Johnson, Inc.

INTRODUCTION

As a woman who has delivered a term infant and who has specified that I plan to feed formula to my infant, I am being invited to enroll my child in a research study of infant formula. My baby and I are being asked to enroll at Truman Medical Center or the University of Kansas Medical Center because the investigators need to know the level of the nutrient studied in my blood and my baby's cord blood after my baby is born. The remainder of the study will be conducted at the University of Kansas Medical Center by Susan Carlson, Ph.D. Approximately 185 subjects will be enrolled in this study.

I do not have to allow my child to participate in this research study. It is important that before I make a decision for my child to participate, I read the rest of this form. I should ask as many questions as I need to understand what will happen if my baby and I participate in the study.

BACKGROUND

Two fats, docosahexaenoic acid (DHA) and arachidonic acid (ARA), are found in very large amounts in the brain. DHA and ARA are important for infant brain development and behavior, including how my baby sees and learning. My baby obtained DHA and ARA from me during the last three months of my pregnancy. Breast feeding is the preferred way to feed in terms of the best interests of the baby. Breast milk also contains DHA and ARA. Breast milk and formulas also contain fats that most babies can change to DHA and ARA.

Infants born early have been shown to have higher development when they consume formulas with DHA or DHA and ARA. This means that preterm infants do not make as much DHA and ARA as they need for best development from the nutrients in infant formula. Term babies (such as my baby) may or may not need DHA and ARA. Some studies indicate they do and others indicate they do not. Some formulas in the US contain DHA and ARA and some do not.

PURPOSE

The purpose of this study is to determine if term infants have higher development when they drink formulas with DHA and ARA. Another purpose is to determine if the amount of DHA and ARA in the formula is important. Human milk DHA can be as low as 0.05% and as high as 2.8%, depending upon a woman's diet. This study will test a range of formula DHA from 0.32% to 0.96% against a formula without DHA or ARA (marketed Enfamil). Infants will be tested for vision, attention (how babies look at faces, look and play with toys), learning, motor and language development.

PROCEDURES

If I choose to enroll my infant in this study after hearing about how the study will be conducted, and what I and my child will need to do, the investigators will record some

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information from my medical record and my delivery including the weight I gained during pregnancy, my smoking history, and my baby's weight, length and head circumference. The investigators will also try to get cord blood for analysis of nutrients in my baby's blood and a sample of my blood when it is drawn after I deliver as part of routine blood work related to my pregnancy. The same nutrients will be analyzed in my blood at the University of Kansas Medical Center.

I will be sent home with an appointment to bring my baby to the University of Kansas Medical Center in about 1 week and given enough marketed Enfamil to feed him/her until that visit (visit 1).

Visit 1 (7-9 days of age) My baby will be weighed and measured. I will be asked what my baby has eaten in the past 24 hours. If I still plan to feed him/her formula, he/she will be assigned by chance (like pulling numbered pieces of paper out of a hat) to one of the following 4 formulas:

- Milk based infant formula containing 0.32% of the total fatty acids as DHA and 0.64% of the total fatty acids as ARA (same as marketed Enfamil Lipil)
- Milk based infant formula containing 0.64% of the total fatty acids as DHA and 0.64%of the total fatty acids as ARA
- Milk based infant formula containing 0.96% of the total fatty acids as DHA and 0.64% of the total fatty acids as ARA
- Milk based Infant Formula without DHA or ARA (same as marketed Enfamil)

I will receive 7 cases of ready-to-feed study formula at this visit. The visit should last about 30 minutes.

Visit 2 (6 weeks of age): The investigators will measure how my baby sees using a test that involves placing 3 electrodes directly on my baby's head. The process involves cleaning the area then placing a small amount of paste similar to toothpaste on the head. The electrodes are placed on top of the paste. The electrodes will be used to record my baby's brain waves while he/she is looking at pictures. My child's weight, height and head circumference will be measured again and I will be asked questions about what my baby eats. I will also be asked questions about my baby's bowel movements including color, number and consistency. I will be asked to report if my baby has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will receive 13 cases of ready-to-feed study formula at this visit. The visit should last about 40 minutes.

Visit 3 (4 months of age): The investigators will measure how my baby sees using the same test as before and another test. My baby will wear a pair of plastic glasses during the second test. My baby's height, weight and head circumference will be measured. In another test, my child will be given an object to look at several times. The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object. My baby's heart rate will be measured during the test.

My baby will have a blood sample collected by either heel stick or drawn from a vein. The investigator may use a cream or spray that will numb the area before obtaining the sample. One-half teaspoon of blood will be drawn.

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I will be asked questions about how much formula my baby drank over the past 24 hours. I will also be asked questions about my baby's bowel movements including color, number and consistency. I will be asked to report if my baby has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will receive 10 cases of ready-to-feed study formula at this visit. The visit will take 60-90 minutes.

Visit 4 (6 months of age): The investigators will measure how my baby sees using the test that requires him/her to where a pair of plastic glasses. In another test, he/she will be given an object to look at several times (just like at 4 months of age). The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object. My baby's heart rate will be measured during the test. My baby's height, weight and head circumference will be measured. I will be asked questions about what my baby eats. I will also be asked questions about my baby's bowel movements including color, number and consistency. I will be asked to report if my baby has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will receive 16 cases of ready-to-feed study formula at this visit. The visit should take 40 -60 minutes.

Visit 5 (9 months of age): My baby will have both tests that measure how he/she sees. In another test, my child will be given an object to look at several times (just like at 4 and 6 months of age). The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object and my baby's heart rate will be measured during the test. My baby's height, weight and head circumference will be measured. I will be asked questions about what my baby eats. I will also be asked questions about my baby's bowel movements including color, number and consistency. I will be asked to report if my baby has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will receive 4 cases of ready to-feed study formula at this visit. The visit should take about 40-60 minutes

Visit 6 (10 months of age): During this visit the baby will be placed on the parent or guardian's lap in front of a small table. A test will be completed with a small toy, foam block and 2 clothes that will be placed in front of the child. The investigator will describe this test to me in detail before it has been completed. I will also take a short language test. The small toy will be given to my child to keep. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will be asked questions about what my baby eats. I will receive 10 cases of ready-to-feed study formula at this visit. I will be asked to bring any unopened cases of study formula to the next visit. The visit should take about 30 minutes.

Visit 7 (12 months of age): I will bring any unopened cases of study formula to this visit. I can feed any cans of formula that remain in an opened case before changing my baby's milk to whole cows' milk. The investigators will measure how my baby sees using both vision tests. My child will be video-recorded while playing with an interesting toy and the investigator will use the recording to measure some aspects of attention. My child will have a blood sample collected by either heel stick or drawn from a vein. The investigator may use a

cream or spray that will numb the area before obtaining the sample. Approximately ½ teaspoon of blood will be drawn. My child's height, weight and head circumference will be measured. I will be asked questions about what my baby eats. I will also be asked questions about my child's bowel movements including color, number and consistency. I will be asked to report if my child has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my child has been sick or not acting well since his/her last visit. The visit should take about 2 hours.

Visit 8 (18 months of age): The investigators will measure how my baby sees using the test that he/she had while wearing plastic glasses. My child will be video-recorded while playing with an interesting toy and the investigator will use the recording to measure some aspects of attention. My child will also be given a standardized test to measure mental and physical development. My child's height, weight and head circumference will be measured. I will be asked questions about what my baby eats. I will be asked questions about my child's language skills. I should let the investigator know if my child has been sick or not acting well since his/her last visit. The visit should take about 2 hours. It is important that my child be rested before the testing at this visit. If for some reason, he/she is not capable of completing all of the assessment, I may be offered the possibility to bring him/her on another day.

RISKS

It is possible that my child could be at risk by participating in this study. Risks of the study formulas may include: not being able to tolerate the formula, spitting up, vomiting, constipation, diarrhea, red itchy skin, rashes or other signs of food allergy and failure to thrive or temporary impairment of growth.

Enfamil Lipil, one of the formulas in this study has been available in stores for the past year. During that year, parent reports of formula problems have been recorded by Mead Johnson Nutritionals, the sponsor of this study. There have not been more problems with Enfamil Lipil than with Enfamil, another formula that will be fed in this study. Two of the formulas have higher DHA and ARA than Enfamil Lipil. None of the formulas fed in this study has more DHA and ARA than has been measured in some human milk, however, higher intakes of DHA and ARA may have some risks that have not yet been identified or unexpected side effects that have not been previously observed.

The importance of DHA and ARA for infants is controversial. Some experts think babies should consume formula with DHA and ARA, others do not. The American Academy of Pediatrics and the FDA have not given the opinion that formulas need to contain DHA and ARA. However, it is possible that my baby might benefit from DHA and ARA and not receive DHA and ARA if he/she is assigned to the formula without DHA and ARA.

Some redness, soreness, or bruising may occur at the site of blood sampling. There is also a very slight risk of infection.

NEW FINDINGS STATEMENT

Any problems of babies in the study will be recorded. I will be informed if any significant new findings develop during the course of the study that may affect my willingness to allow my child to participate in this study.

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BENEFITS

My child may or may not benefit from participating in this study. It is hoped that additional information gained in this research study may be useful in the growth and development of infants. I will receive a video recording of my infant doing the 4, 6 and 8 month looking test when the 8 month visit is complete.

ALTERNATIVES

Formulas with no DHA and ARA and formulas with the lower level of DHA and ARA in this study are available in stores and from WIC (Women Infant Children Supplemental Feeding Program). Name brand formulas that contain DHA and ARA are Enfamil Lipil and Similac Advance. Name brands that do not contain DHA and ARA are Enfamil and Similac. As noted above, two of the formulas fed in this study are the same as Enfamil and Enfamil Lipil. The other formulas contain 2 and 3 times as much DHA as Enfamil Lipil and the same amount of ARA. Store brands of formula are also available locally (for example, Costco, Walmart) without DHA and ARA.

COSTS

Infant formula will be provided to me at no cost while my child is participating in this study. The investigators will work with WIC at Truman Medical Center to make sure that I receive baby foods other than formula until my baby is 12 months old. I will not incur any costs because of my child's participation.

PAYMENT TO SUBJECTS

I will receive a check for $50 at each visit to the University of Kansas Medical Center to cover the costs of transportation and to partially compensate me for my time required to participate in the study. There will be 8 regularly scheduled visits in 18 months. If an additional visit is required because my infant is unable to complete all of the testing at 18 months, I will receive an additional payment of $50 for another visit.

My name, address, social security number, and the title of this study will be given to the KUMC Research Institute. This is done so that the Research Institute can write a check for study payments. Payments are taxable income.

DISCLOSURE OF FINANCIAL INTERESTS

The principal investigator has been paid as a consultant and for program presentation on DHA for Mead Johnson Nutritionals (the sponsor). The University of Kansas Medical Center Conflict of Interests Committee monitors this research project to make it less likely that these financial interests inappropriately influence how the study is conducted. However, you should make your own decision about whether these financial interests affect your decision to participate. If you have any questions about this financial relationship, you may discuss them with the investigator or with the Research Compliance division at 913-588-5492.

IN THE EVENT OF INJURY

In the event my child experiences any serious health problem (hospitalization, life-threatening illness or death) for any reason while participating in this study, I should immediately seek treatment or help in the way I normally would as if my child were not in a study. I should let

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Susan Carlson, Ph.D. know about any of these problems as soon as possible by calling her office (913-588-5359) between 8:30 and 5:30 Monday through Friday. If it is after 5:30 PM on a weekday, or it is a holiday or weekend, I should call Dr. Carlson at home (816 -960-1805). A message may be left at both numbers in the event that Dr. Carlson is not immediately available.

INSTITUTIONAL DISCLAIMER STATEMENT

Although the University of Kansas Medical Center does not provide free medical treatment or other forms of compensation to persons injured as a result of participating in research, such compensation may be provided under the terms of the Kansas Tort Claims Act. If I believe my child has been injured as a result of participating in research, I should contact the Office of Legal Counsel, University of Kansas Medical Center, Kansas City, KS 66160-7101. I do not give up any of my or my child's rights by signing this form.

It is not the policy of the University of Missouri nor Truman Medical Center to compensate human subjects in the even the research results in injury. The University of Missouri and Truman Medical Center, in fulfilling their public responsibilities, have individually and separately provided liability coverage for any physical injury in the event such injury is caused by the negligence of the University of Missouri, its faculty or staff or Truman Medical Center and its employees. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to subjects who suffer injuries as a result of participating in the research projects of the University of Missouri. In the event I believe that I have suffered any physical injury as the result of my participation in the research program, I may contact Dr. Susan Carlson, 913-588-5359, or Sheila Anderman, Research Administrator of the University of Missouri-Kansas City Adult Health Sciences Institutional Review Board, telephone number 816-235-6150, who can review the matter with me and provide further information on how to proceed.

CONFIDENTIALITY AND PRIVACY AUTHORIZATION

Names of subjects or information identifying subjects will not be released without written permission unless required by law. Study data will be recorded on the sponsor's forms and sent to the sponsor or their designee. Videotapes of my baby when he/she is looking at pictures and playing with toys will be used only by the investigators and their students and to make a videotape copy for me. The videotapes will be secured under lock and key like all of other information that could be linked directly to my child. The videotape of my child will not be shown without specific permission from me and even then would not identify my child by name. Efforts will be made to keep my and my child's personal information confidential. Researchers cannot guarantee absolute confidentiality. If the results of this study are published or presented in public, information that identifies my baby will be removed.

The privacy of my and my child's health information is protected by a federal law known as the Health Insurance Portability and Accountability Act (HIPAA). If I choose to allow my child to participate in this study, I will be asked to give permission for researchers to use and disclose my and my baby's health information that is relevant to the study.

To perform this study, researchers will collect health information about me and my child from his/her medical record and from the study activities that are listed in the Procedures section of this consent form. My and my baby's study-related health information will be used at KU

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Medical Center by Dr. Carlson, members of the research team, Truman Medical Center, the KU Hospital Medical Record Department, the KUMC Research Institute and officials at KUMC and at Truman Medical Center that oversee research, including the KUMC Human Subjects Committee, the IRB that governs Truman Medical Center and other committees and offices that review and monitor research studies.

Dr. Carlson and her team may share information about me and my baby with representatives of Mead Johnson (the sponsor of the study), the monitoring company who verifies study data, the laboratory that processes study lab samples, other business partners of the sponsor who help with the study, Mead Johnson's Data Coordinating Center, Mead Johnson's designated Data and Safety Monitoring committee, the U.S. Food and Drug Administration (FDA), and U.S. agencies that govern human research (if and when regulatory compliance issues arise). My and my child's information will be shared in order to analyze and confirm the results of the study.
Some of the persons or groups that receive my and my baby's study information may not be required to comply with HIPAA privacy laws. My and my child's information may lose its federal protection if those persons or groups disclose it.

Permission granted on this date to use and disclose my health information remains in effect indefinitely. By signing this form I give permission for the use and disclosure of my and my child's information for purposes of the study at any time in the future.

Any research information that is placed in my and my child's medical record will be kept indefinitely.

QUESTIONS
I have read the information in this form. Dr. Carlson or her associates have answered my question(s) to my satisfaction. I know if I have any more questions after signing this I may contact Dr. Carlson or one of her associates at (913) 588-5359. If I have any questions about my child's rights as a research subject, I may call (913) 588-1240 or write the Human Subjects Committee, University of Kansas Medical Center, 3901 Rainbow Blvd. MSN 1032, Kansas City, KS 66160.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY
My and my child's participation in this study is voluntary and that the choice not to participate or to quit at any time can be made without penalty or loss of benefits. Not participating or quitting will have no effect upon the medical care of treatment my child receives now or in the future at the University of Kansas Medical center. The entire study may be discontinued for any reason without my consent by the investigator conducting the study, by the sponsor of the study, or the FDA. My child's participation can be discontinued by the investigator or by the sponsor if it is felt to be in my child's best interest or if I do not follow the study requirements. If I choose to withdraw before my child is 18 months of age, I may be asked to answer questions about the study on the telephone.

If I want to cancel permission to use my or my child's health information, I should send a written request to Dr. Carlson. The mailing address is Susan Carlson, Ph.D., Dept. of Dietetics and Nutrition, 4019 Delp, University of Kansas Medical Center, 3901 Rainbow

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Boulevard, Kansas City, KS 66160. If I cancel permission to use my child's health information, the research team will stop collecting any additional information about my child, unless they need information about a side effect of the milk-based formula. The information that was collected before my cancellation, and any information about side effects, will be sent to the study sponsor.

Should the study be terminated prior to the completion of my and my child's participation, neither the sponsor, the investigator, nor the University of Kansas Medical Center will be under any obligation to provide me with the milk-based formula used in the study. My child's physician will decide upon further treatment after study termination, if indicated.

CONSENT

Dr. Carlson or her associates have given me information about this research study. They have explained what will be done and how long it will take. They explained the inconvenience, discomfort and risks that may be experienced during this study.

By signing this form, I give my permission for my and my child's health information to be used and disclosed for the purposes of this research study. If I choose not to sign this form, my child and I will not be able to participate in the study.

I voluntarily consent to allow my child and I to participate in this research study. I have read the information in this form and have had an opportunity to ask questions and have them answered. ***I will be given a copy of the signed form to keep for my records.***

_____
Type/Print Subject's Name

_____ _____ _____
Signature of Subject Time Date

_____
Type/Print Name of Witness

_____ _____
Signature of Witness Date

_____
Type/Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

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Appendix G

Parent Study Consent Form: 2 to 6 Years of Age

CONSENT FORM

The Effects of Infant Formula Supplemented with Long Chain Polyunsaturated Fatty Acids on Cognitive Development in Children

Protocol #10205
Sponsor: Mead Johnson, Inc.

INTRODUCTION

As a parent who enrolled my child in a study of infant formula between birth and 18 months, I am being asked if I will permit my child to be studied with more tests of infant development at 7 more ages (9 more times) ending when he/she reaches 6 years of age. The study will be conducted at the University of Kansas Medical Center by Susan Carlson, Ph.D. and other members of her study team. Up to 110 children will be studied.

I do not have to allow my child to participate in this research study. It is important that before I make a decision for my child to participate, I read the rest of this form. I should ask as many questions as I need to understand what will happen if my baby and I participate in the study.

BACKGROUND

Two fats, docosahexaenoic acid (DHA) and arachidonic acid (ARA), are found in very large amounts in the brain. DHA and ARA are important for infant brain development and behavior, including how my baby sees and learning. My baby was enrolled in a study that provided varying amounts of DHA and ARA when he/she was an infant. Until 18 months, my infant/toddler was followed for his/her development. Now the investigator (Dr. Carlson) has been given additional money to follow children from that study until they are 6 years of age.

PURPOSE

The purpose of the original study was to determine if term infants have higher development when they drink formulas with DHA and ARA. Another purpose was to determine if the amount of DHA and ARA in the formula is important. My baby had tests of, vision, attention (how babies look at faces, look and play with toys), learning, motor and language development. These are still the purposes of the study. This new consent would permit the investigators to continue studying my child's development until he/she was near school age. Child development experts believe that any benefits of formulas with DHA and ARA would become bigger as children became older.

PROCEDURES

If I choose to enroll my infant in this study after hearing about how the study will be conducted, and what I and my child will need to do, I will be given an appointment to bring my child in when he or she is 2 years old. At the 2-year appointment and all subsequent appointments, it is important that my child not be tired or sick so that he/she can do his/her best. The investigators will work with me to find a time of day that is a good one for his/her appointment.

Visit 1 (2 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. I will be asked to complete a questionnaire about my child's experiences and environment. During

this visit the child will sit in a toddler chair in front of a small table. A test will be completed with a small toy, in which the toy is hidden in one of two places in front of the child. My child will also be shown how to put together small toys and will be given a chance to do so. In addition, my child will play with interesting toys. The investigator will describe these tests to me in detail before each is started. This visit will be videotaped and I will be with my child the whole time. I will also complete a survey about my child's everyday behavior. This visit will take approximately 1.5 to 2 hours.

Visit 2 (2.5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. During this visit the child will sit in a chair in front of a small table. A test will be completed with a small toy, in which the toy is hidden in one of two places in front of the child. My child will also be shown how to put together small toys and will be given a chance to do so. In addition, my child will play with interesting toys. The investigator will describe these tests to me in detail before each is started. This visit will be videotaped and I will be with my child the whole time. This visit will take approximately 1.5 hours.

Visit 3 (3 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. During this visit the child will sit in a chair in front of a small table. A test will be completed with a small toy, in which the toy is hidden in one of two places in front of the child. In addition, my child will be shown cards and asked for a response to them or asked to sort them into piles. The investigator will describe these tests to me in detail before each is started. This visit will be videotaped and I will be with my child the whole time. This visit will take approximately 1.5 hours.

Visit 4 (3.5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. During this visit the child will sit in a chair in front of a small table. A test will be completed with a small toy, in which the toy is hidden in one of two places in front of the child. In addition, my child will be shown cards and asked for a response to them or asked to sort them into piles... The investigator will describe these tests to me in detail before each is started. The visit will be videotaped and I will be with my child the whole time. This visit will take approximately 1.5 hours.

Visit 5 (4 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. In addition, my child's blood pressure will be taken. During this visit the child will sit in a chair in front of a small table. My child will be given a set of cards and asked to sort them into piles and will play a game in which monkeys will be placed in a tree according to a few rules. My child will be given a set of cards and asked to sort them into piles or give a certain response to a card. In addition, my child will be shown and set of pictures in a certain order and will be given a chance to put those pictures in order. The investigator will describe these tests to me in detail before each is started. The visit will be videotaped and I will be with my child the whole time. This visit will take approximately 1.5 hours.

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Visit 6 (4.5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. I will be asked to complete a questionnaire about my child's experiences and environment. My child's blood pressure will be taken. During this visit, 24 sensors will be placed on my child's head and 2 additional sensors will be placed on my child's chest. This procedure involves using a cotton swab to gently clean the area where each sensor will be placed and then sticking the sensor in place using a paste that washes out with water. After the sensors are in place, my child will be shown how to play a computer game in which buttons are pressed when certain pictures come up on a television screen or will be asked simply to watch pictures on the television. My child's brain activity and heart rate will be recorded during the computer games. The investigator will describe these tests to me in detail before each is started. I will be with my child the whole time. You will be asked some questions about your child's health and home life. This visit will take approximately 1.5 hours.

Visit 7 (5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. In addition, my child's blood pressure will be taken. During this visit the child will sit in a chair in front of a small table. My child will be given a set of cards and asked to give a certain response to a card and will play a game in which monkeys will be placed in a tree according to a few rules. In addition, my child will be shown and set of pictures in a certain order and will be given a chance to put those pictures in order. The investigator will describe these tests to me in detail before each is started. The visit will be videotaped and I will be with my child the whole time. In addition, my child will be given a test of language abilities. You will be asked some questions about events in your child's life and his or her behavior. This visit will take approximately 1.5 hours.

Visit 8 (5.5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. My child's blood pressure will be taken. During this visit, 34 electrical sensors will be placed on my child's head and 2 additional sensors will be place on my child's chest. This procedure involves using a cotton swab to gently clean the area where each sensor will be placed and then sticking the sensor in place using a paste that washes out with water. After the sensors are in place, my child will be shown how to play a computer game in which buttons are pressed when certain pictures come up on a television screen or will be asked simply to watch pictures on the television. My child's brain activity **and heart rate** will be recorded during the computer games. The investigator will describe these tests to me in detail before each is started. I will be with my child the whole time. You will be asked some questions about your child's health and home life. This visit will take approximately 1.5 hours.

Visit 9 (6 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. In addition, my child's blood pressure will be taken. During this visit, my child will sit in a chair in front of a small table. My child will be play a game in which monkey's will be placed in trees according to a few rules. In addition, my child will also be asked to play with blocks, put puzzles together, and be asked questions to test their general knowledge, comprehension, and vocabulary. My child will also be presented with different patterns or shapes and be asked to fill in the missing piece. In addition, my child will be shown a series of pictures and

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be asked which two go together. My child will also be shown a series of symbols and be asked to find the two that match. My child will play a game and try to figure out what the investigator is thinking of based on the clues given. My child will also be shown a series of pictures and be asked what is missing. The investigators will describe these tests to me in detail before each is started. The visit will be videotaped and I will be with my child the whole time. I will be asked some questions about events in my child's life. This visit will take approximately 2 hours.

RISKS
There are no known risks from any of the tasks that my child will be asked to do. Some of the tasks may be tiring and my child may not like wearing the cap, but the investigators will not continue with a test if the child is not performing at his/her best because he/she is tired or excessively bothered by wearing the cap.

NEW FINDINGS STATEMENT
The study will continue to follow the development of my child between 2 and 6 years of age. I will be informed if any significant new findings develop during the course of the study that may affect my willingness to participate or to allow my child to participate in this study.
I may request to know results when the study is complete.

BENEFITS
My child will not benefit from participating in this study. It is hoped that additional information gained in this research study may be useful in the growth and development of infants.

ALTERNATIVES
My child does not have to participate in this research study.

COSTS
I will not incur any costs because of my child's participation.

PAYMENT TO SUBJECTS
I will receive a check for $100 at each visit to the University of Kansas Medical Center to cover the costs of transportation and to partially compensate me for my time required to participate in the study. If I do not have enough money to come for the visit, I may ask the investigators to pay for a cab to and from the appointment andI will be given the $100 check, however, the investigators will have to deduct the cost of the cab from my next check. There will be 8 regularly scheduled visits in 4 years. If an additional visit is required because my infant is unable to complete all of the testing at 6 years of age, I will receive an additional payment of $50 for another visit.

My name, address, social security number, and the title of this study will be given to the KUMC Research Institute. This is done so that the Research Institute can write a check for study payments. Payments are taxable income.

DISCLOSURE OF FINANCIAL INTERESTS
The principal investigator has been paid as a consultant and for program presentation on DHA for Mead Johnson Nutritionals (the sponsor). The University of Kansas Medical Center

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Conflict of Interest Committee monitors this research project to make it less likely that these financial interests inappropriately influence how the study is conducted. However, you should make your own decision about whether these financial interests affect your decision to participate. If you have any questions about this financial relationship, you may discuss them with the investigator or with the Research Compliance division at 913-588-5492.

INSTITUTIONAL DISCLAIMER STATEMENT

If you believe you have been injured as a result of participating in research at Kansas University Medical Center (KUMC), you should contact the Director, Human Research Protection Program, Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. Compensation to persons who are injured as a result of participating in research at KUMC may be available, under certain conditions, as determined by state law or the Kansas Tort Claims Act.

CONFIDENTIALITY AND PRIVACY AUTHORIZATION

Names of subjects or information identifying subjects will not be released without written permission unless required by law. Study data will be shared with the sponsor, but I will not be identified. Videotapes of my baby when he/she is looking at pictures and playing with toys will be used only by the investigators and their students. The videotapes will be secured under lock and key like all of other information that could be linked directly to my child. The videotape of my child will not be shown without specific permission from me and even then would not identify my child by name. The videotapes will be destroyed after all of the study data are collected and analyzed. Because study will continue for 4 more years and enrollment occurred during 2 years, the investigators may keep a copy of my child's videotape for as long as 8 years. Efforts will be made to keep my and my child's personal information confidential. Researchers cannot guarantee absolute confidentiality. If the results of this study are published or presented in public, information that identifies my baby will be removed.

The privacy of my and my child's health information is protected by a federal law known as the Health Insurance Portability and Accountability Act (HIPAA). If I choose to allow my child to participate in this study, I will be asked to give permission for researchers to use and disclose my and my baby's health information that is relevant to the study.

Because this is a continuation of an existing study, researchers already have some health information about my child from his/her medical record with consent. They will not obtain any other information except the information that they conduct as shared in the Procedures section. My baby's study-related health information will be used at KU Medical Center by Dr. Carlson, members of the research team, the KU Hospital Medical Record Department, the KUMC Research Institute and officials at KUMC that oversee research, including the KUMC Human Subjects Committee, and other committees and offices that review and monitor research studies.

Dr. Carlson and her team may share information about me and my baby with representatives of Mead Johnson (the sponsor of the study), the U.S. Food and Drug Administration (FDA), and U.S. agencies that govern human research (if and when regulatory compliance issues

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arise). My and my child's information may be shared in order to analyze and confirm the results of the study.

Some of the persons or groups that receive my and my baby's study information may not be required to comply with HIPAA privacy laws. My and my child's information may lose its federal protection if those persons or groups disclose it.

Permission granted on this date to use and disclose my health information remains in effect indefinitely. By signing this form I give permission for the use and disclosure of my and my child's information for purposes of the study at any time in the future.

QUESTIONS

I have read the information in this form. Dr. Carlson or her associates have answered my question(s) to my satisfaction. I know if I have any more questions after signing this I may contact Dr. Carlson or one of her associates at (913) 588-5359. If I have any questions about my child's rights as a research subject, I may call (913) 588-1240 or write the Human Subjects Committee, University of Kansas Medical Center, 3901 Rainbow Blvd. MSN 1032, Kansas City, KS 66160.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY

My and my child's participation in this study is voluntary and that the choice not to participate or to quit at any time can be made without penalty or loss of benefits. Not participating or quitting will have no effect upon the medical care of treatment my child receives now or in the future at the University of Kansas Medical center. The entire study may be discontinued for any reason without my consent by the investigator conducting the study or by the sponsor of the study. My child's participation can be discontinued by the investigator if I do not come for scheduled visits.

You have a right to change your mind about allowing the research team to have access to your healthy information. To cancel your permission you must send a written request to Dr. Carlson at the University of Kansas Medical Center, Dept. of Dietetics and Nutrition, Mail Stop 4013, 3901 Rainbow Boulevard, Kansas City, KS 66160. If you cancel permission to use your health information, you will be withdrawn from the study and the researchers will stop collecting information about you. The researchers and the sponsor may continue to use and share information that was gathered before your cancellation.

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CONSENT

Dr. Carlson or her associates have given me information about this research study. They have explained what will be done and how long it will take. They explained the inconvenience, discomfort and risks that may be experienced during this study.

By signing this form, I give my permission for my child to continue with followup for an additional 8 visits (at 6 ages) between 2 and 6 years of age. If I choose not to sign this form, my child and I will not be able to participate in the study.

I voluntarily consent to allow my child and I to participate in this research study. I have read the information in this form and have had an opportunity to ask questions and have them answered. ***I will be given a copy of the signed form to keep for my records.***

_____
Type/Print Subject's Name

_____ _____ _____
Signature of Subject Time Date

_____
Type/Print Name of Witness

_____ _____
Signature of Witness Date

_____
Type/Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

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